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SIMPLE SYSTEMS FOR THE STUDY OF LEARNING MECHANISMS

A report of an NRP Work Session

chaired by

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SIMPLE SYSTEMS FOR THE STUDY OF LEARNING MECHANISMS

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by

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C O N T E N T S

(Staff Editors: J. Carolyn Register
and Anne H. Rosenfeld)

INTRODUCTION

The amazing phenomena subsumed under "learning," all-important in the later stages of the evolution of life and in man's struggles to solve his problems, have been objects of close study for decades (but only for decades!) by experimental and theoretical psychologists. In the last few years an explosion of interest and effort has been directed towards revealing something of the mechanisms responsible at physiological and lower levels.

It should not be surprising or discouraging that the result today, and doubtless for years to come, is a bag of eels -- a slippery assortment of data and interpretation that is now questionably relevant, now dubiously established, and at best difficult to evaluate. Learning surely represents one of the highest mysteries of nature. Taking learning together with other aspects of human and animal behavior, and considering the corollary achievements of its substrate, the nervous system (e.g., in sensory discrimination, recognition, motor coordination, etc.), we may regard that system with some awe. Certainly the behavioral machine is the most complex of natural systems, but for systems of such systems, i.e., social groups. The nervous mechanism thus occupies a unique position in accounting for the manifestations of life. Although difficulty in interpreting fragments of the picture is to be expected by its nature, serious research effort is justified by the significance of the problem.

The explosion of interest in a deeper understanding of the processes of learning cannot wait for advances in basic neurophysiology to provide adequate insight. Work on several levels must go forward simultaneously. Because of the inherent intricacy of the problem, an understandable stratagem, although fraught with its own difficulties, is the widespread effort to analyze the simplest examples of learning. One hopes thereby to avoid much of the adventitious and corollary complexity of advanced examples, to reduce the variability that might accompany greater elaboration, or, in any case, to find basic common denominators of the mechanisms as distinct from superimposed and derived features. What follows will illustrate how rocky is the path even to such relatively modest goals.

Like other Work Session reports, the present summary attempts neither a systematic or didactic account of what is known nor a review of the relevant literature. Instead, this report reflects the aims of the Work Session itself: to

examine the heuristic opportunities presented by a number of promising preparations or simplified systems, to examine the results and conceptual questions that experimental design can illuminate, and thus indirectly to stimulate the discovery of new and favorable materials for the study of mechanisms underlying learning.

The Work Session as a medium does not generally permit organized or comprehensive treatment and its scintillae are peculiarly elusive. The mode of harvesting attempted here is a blend of narrative reporting and selective exposition, strongly influenced by subjective factors in the abstracting, writing and editing. To this the NRP Staff contributed greatly but THB must be held accountable.

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I. THE COMPARATIVE STUDY OF LOWER ANIMALS

The strategy of analyzing learning in simple systems involves two broad classes of tactics: one may look at instances of learning in intact, relatively simple animals, or in fragments or simplified systems of either lower or higher animals. This section deals with the first of these alternatives.

We should like to know several things: Is there learning and if so what is it like in lower forms? What species are likely to be favorable for closer study? In what ways has learning evolved? Can one part of the nervous system be implicated in particular? Partial answers to all these questions are available though they are not explicit or specific enough to be satisfying.

Neither the Work Session nor this report can pretend to review the literature, especially concerning comparative studies of lower groups. The interested reader is referred to Warden, Jenkins and Warner (1936), Thorpe (1963a), Thorpe and Davenport (1965), and the bibliographies of these works. A selective survey of some of the available information is given below, following some extracts of the Work Session discussion.

A major conclusion of the Work Session, reinforced by testimony from various authors, is that we would be mistaken if we expected to find some sort of unitary function representing learning in all phyla and all organisms. All organisms do show, to a greater or lesser extent, adaptability to their environment, an ability to modify behavior adaptively in response to certain kinds of stimulation; but the kinds of modifiability available to them differ. It is still an open question whether there are only a few or a good many different basic kinds of learning, especially in terms of the mechanisms involved. However, habituation, sensitization, reactive inhibition, associative hysteresis, conditioned inhibition, avoidance conditioning, instrumental conditioning, classical conditioning, one-trial learning, insight learning, and imprinting, to name some of the phenomena that invite the name "learning," are so diverse in character that the assumption of a single mechanism seems unjustified without positive evidence. Obviously, intensified comparative study in this area is needed.

Chorover pointed out the <u>importance of comparative study of ethology</u> which, in addition to its intrinsic interest, provides clues to the behavioral repertoire of the several species that should dictate the kind of experimental investigations to

be done. Each organism possesses certain behavioral limitations set by its performance capabilities; perhaps some animals are more suitable than others for the study of neural and biochemical mechanisms in learning.

To Chorover, trying to define a certain phylogenetic point at which learning first begins is like trying to decide at which point a child begins to think. Both tasks are difficult, if not impossible.

Even if one cannot identify a particular stage in evolution at which learning began, it can be asserted that learning evolved toward a greater role in behavior in higher forms, speaking broadly of the whole range of animal groups. Questions about the meaning of this generalization were raised by several Work Session participants. Generally, however, they were not answered, since the available factual knowledge of a comparative nature, though bulky, is inadequate for many animal groups and is often not really comparable in diverse animals. We need new data on each of the types of learning, with tasks chosen quite knowingly for the given species. Do lower forms need more trials than higher? Do they tend to generalize more? Do they extinguish more quickly? Can they learn fewer discriminations? Along what dimension do the tasks that higher animals can perform increase in difficulty?

Eisenstein asked, as an example of the problem confronting us in this area: How does one compare the learning ability of an earthworm with that of a rat? Since their receptor and effector mechanisms are not at all alike, it is difficult to differentiate between what represents learning ability per se and what represents the response limitations imposed by a given receptor and effector system. He suggested the use of electrophysiological endpoints, equating the different organisms in terms of electrical input and spike response down an output nerve. Having equated the input and output parameters, one could ask how the integration of these two occurs as a function of a number of manipulations. Then, by systematically using the procedures for producing generalization, discrimination, extinction, habituation, and learning, one could observe how these principles change as a function of increasing structural complexity.

Bullock raised the question whether there is a phylogenetic trend in the degree to which the nervous system works by deterministic as opposed to a probabilistic control. He believes there is a great deal of each form of control. The stretch-receptor neuron and its inhibitor in the crayfish, for

example, cannot be very probabilistic since every inhibitory impulse that arrives has a decisive effect with only a small amount of play. But in other systems where the behavior of the single unit is less reliable than the complete behavior -- say a pianist reading a note, or a dog responding to a learned tone -- the total behavioral response cannot be predicted from the response of any unit we know of now; it then seems proper to speak of a probabilistic determination of the output. Bullock thinks that learning takes place in more than one of these classes; since mechanisms might be different in the simple deterministic system and in a probabilistic system, different approaches might be required to analyze them. This raises the whole crucial and complex problem of localization of learning, i.e., of finding the place worth analyzing.

Chorover felt there would be more deterministic systems in lower organisms. Asked by Tauc whether he considers a plastic system more unpredictable, Bullock replied that plasticity does not mean unpredictability; it might be built in as a consistent property.

Schmitt asked whether there is any structural characteristic not seen below a certain invertebrate level that might indicate roughly the boundary within which a certain kind of learning occurs. Strumwasser suggested that as a general characteristic, complexity of neuronal branching may parallel learning ability. Bullock added that the flatworms may be strategically significant because a drastic change in type of neuron and histological organization occurs in them as compared to simpler groups; one might thus expect a comparable change in level or type of learning. Cohen questioned the significance of the ganglionated aspect of structural evolution such as first appears in the flatworm.

A question related to the development of memory in lower groups concerns the extent of mitotic stability among neurons. Do we know whether neurons of coelenterates are undergoing mitosis, where in phylogeny mitotic stability begins, and whether it has something to do with learning? In fact do we really know that neurons in higher organisms are not undergoing mitosis? We know that in lower animals, mitosis in the CNS occurs at those postnatal stages that are just a continuation of the individual's development. In earthworms, the cells in the brain increase in number for weeks after hatching, and then stabilize. But even in mammals, the new work of Altman (1967) (using uptake of labeled thymidine) shows considerable neurogenesis even in the adult brain, including areas like the hippocampus (implicated in memory processes). Considerable

plasticity exists in the numbers of neurons found in a given nucleus under influences such as growth hormone or environments impoverished or enriched in sensory stimuli.

Finally, during the general discussion, Edelman asked whether any inbred "idiot insects" exist that might be useful tools for study. Cohen reported that Manning (personal communication), in Edinburgh, is inbreeding "fast" and "slow" cockroaches designated according to their motor tendencies. Caution, of course, is required because it is well-known that certain tasks that we might set are unsuitable and cannot be learned. Dogs, for example, seem very bright when asked to learn that a tone of 1000 cps means food and another, very slightly different, means none, but appear stupid if asked to learn that 1000 cps means food in a certain place in the room and a considerably different tone means food in a different place (Konorski). Rats readily learn the sequence of turns right, right, left, left, in a spatial maze but cannot learn this task in very many more trials in a temporal maze (Hunter, see Woodworth and Schlosberg, 1964). We do not have universal test tasks that are "fair," nor do we have sufficient insight into given species to know what tasks each should be able to solve. A basic difficulty thus exists in comparing learning ability especially as between different families, orders, and higher categories.

Protozoa

Turning to a brief survey of data on selected groups of animals, some remarks may first be made on the Protozoa. More extended reviews of the evidence for nervous structures and for learning are to be found in Bullock and Horridge (1965), McConnell (1965), Gelber (1965), and Jensen (1965). In sum, there is no satisfactory evidence for structures specialized for conduction or coordination of behavior (mainly ciliary locomotion). But there is evidence of learning, defined broadly (see Section VII, below) even after discounting many reports. Plavilstshikov (1928) reported that the colonial ciliate *Carchesium* could be conditioned by pairing touch and light for an average of 139 $\pm$ 25 trials. Strict controls of all the types recognized in Section VIII, below, may not be fully satisfied but the case cannot now be dismissed. Older studies showing, for example, adaptive increase in body-wall flexibility, conform to virtually all recent definitions of learning, though of course not to conditioning. Habituation is described in recent unpublished reports on *Stentor coeruleus* (C.M. Harden, Scientific Engineering Institute, Waltham, Mass.) and *Spirostomum* (P.B. Applewhite, Yale Univ.). Jennings' (1915) classical work and that of other pioneer authors should not be

overlooked, for mixed with behavioral changes attributable to environment or to fatigue and the like, is evidence of internal change. To Bullock, the case for behavioral alteration in Paramecium lasting for 10 hours or more, and not attributable to environment, injury, or the other exclusions listed below (see p. 182), seems stronger than ever after the trials by fire in recent years. Under suitable conditions, Paramecium shows "reactive inhibition" when forced several times to turn to the same side at the choice point in a narrow T-shaped channel. That is, it tends to respond to a free choice by turning toward the other, non-forced side. Explanation of this behavior requires the assumption of some kind of memory (Rabin and Hertzler, 1965).

Gelber's (1965) experiments appear to demonstrate learning in some form (as in Section VII, below) though it is hard to specify which. Her basic experiment depends on the tendency of Paramecium to avoid a clean platinum wire and to aggregate at a source of bacterial food. Repeatedly dipping a platinum wire baited with bacteria into the ciliate culture constitutes a series of trials. After 15 trials, a clean wire dipped into the culture attracts Paramecium up to and beyond 10 hours later. Controls seem to have accounted for the imaginable possibilities of environmental change. However, since one feels less rapport with these animals, one can be less confident that all desirable controls have been thought of. Without specific reasons, some of the Work Session participants were unwilling to take the position that pro tem a case has been made for a property related to learning in protozoans; others, however, were willing and the problem as in the next few groups became a subjective matter of betting on horses.

Coelenterates

The coelenterate nervous system, the simplest known, has invited much anatomical and physiological study (Bullock and Horridge, 1965). But there is remarkably little work directed at the question: Can coelenterates learn? Davenport recounted some recent experiments of Ross (1965) on conditioned inhibition in sea anemones. Stomphia coccinea has a species-characteristic response to contact by the sea star, Dermasterias imbricata; it releases its hold of the substratum and embarks on an extraordinary bout of swimming movements. It also responds to mechanical prodding of its base by closing and contracting down onto the substratum. Pairing these two stimuli a number of times (with starfish material first and 2 seconds later a basal disc prod), and then testing with starfish material alone, none of eight animals showed a swimming response on the first

test trial. Instead, they contracted, and only slowly resumed the swimming response as test trials were repeated. Neither random sequence nor reverse sequence of the two stimuli were used as controls, but Davenport was impressed by the evidence of plasticity and relative complexity in a form with only a diffuse nerve net. Bullock added that older evidence of spontaneous changes of "mood" or "set" in coelenterates similarly argues for some degrees of freedom beyond a mere reflex net. There is, furthermore, strong evidence of habituation in Hydra (Rushforth, 1965). Davenport underlined the considerable degree of specificity of recognition and response in the sea anemone Calliactis parasitica, which detects passing hermit crabs, exchanges information between its foot and tentacles, releasing its foot, reattaching its foot to the crab shell, and then releasing its tentacles. No learning need be invoked at present, but this preparation may be worth further study.

Another preparation is Stoichactis, an anemone that commonly has several specimens of a small species of fish living among its tentacles. These fish are not stung by their host but if they are brought into contact with almost any other kind of anemone, they will be seized, stung, and frequently eaten. Likewise, Stoichactis will seize and sting any other fish that come into contact with it, including, at times, fish of the partner species that have been maintained in isolation from the anemone ("unacclimated" fish). Davenport and Norris (1958) have demonstrated that mucus from the fish raises the threshold of discharge of the host's nematocysts. It has been claimed by some observers that the host anemone "recognizes" not just any fish in the privileged species, but only those individuals that have been living in association with it. However, controlled experiments demonstrating this are not at hand. McCleary thought this a critical point, because if it is the species that is recognized, one might assume an innate predisposition exists, but if recognition takes place on an individual basis, it might be an instance of learning. The current researches of Mariscal (1965) have given no evidence that a change is required in the anemone during the process that has occurred when an unacclimated fish, after making contact gingerly with its host, finally is no longer stung. It would appear that only a reflex change in skin secretion in the fish is necessary for protection to occur. However, unpublished studies by Blosch seem to indicate that in the establishment of some anemone-fish partnerships, a "change-of-state" in the anemone may be involved.

To date, no evidence is available that the sort of long-term storage of previous experience that may be defined

as learning exists in coelenterates; if such a phenomenon as conditioning exists, it should be looked for among forms with as specific and yet complicated behavior as Calliactis or Stomphia.

Platyhelminthes

Flatworms, especially fresh-water triclads of the group of planarians, have been objects of much attention and controversy in the last few years. The reviews of McConnell (1965) and Jacobson (1963, 1965) are very useful, although written by protagonists; critiques by Jensen (1965) and Bennett and Calvin (1964) do not deal with the bulk of the literature and in many respects are more vulnerable than the first named. Older experiments are reviewed in Warden, Jenkins, and Warner (1936), Hyman (1951), and Bullock and Horridge (1965), except for an important rediscovered paper by van Oye (1920). The details were beyond the scope of the Work Session and had been examined to some extent at previous N.R.P. conferences. No review will be attempted in this report, but only a few summarizing statements as seen by the Chairman.

First, in respect to histologically differentiated ganglia, sense organs, and behavioral repertoire, planarians are rather poorly endowed compared to their marine relatives, the polyclad flatworms. But it may be asserted safely that planarians show environmentally induced alterations in behavior (not attributable to injury, growth, or acclimation) that persist, with high statistical significance, for hours, days, and even weeks. The demonstration of habituation, reactive inhibition (spontaneous alternation after forced choice of direction), and conditioned inhibition ("conditioned lethargy") in them is hardly to be doubted. Instances of classical and instrumental conditioning have repeatedly been described, and at high levels of statistical significance; but attempts at replication have not always been successful. As with other animals remote from ourselves, it is difficult to think of all the conditions that may be important. For example, it has gradually come to light that planarians may behave erratically in laboratory-cleaned ware and in small dishes; may exhibit circadian and possibly semilunar or lunar cycles of reactivity, interest in food, and "teachability;" may have significant species differences; may react quite differently to some forms of punishment (electric shock with a component of polarization) according to their orientation in the field at the moment; and may abruptly reverse the sign of some responses. It is understandable, then, that results have not been consistent and that we do not yet appreciate all the environmental, or internal, factors that influence these creatures.

The experiments reported on transfer of training by cannibalism upon trained worms are more difficult to summarize and/or evaluate. Positive results may represent not specific transfer but rather, generalized activity-level or "set" effects.

Annelids

Annelids offer some of the most promising material for study of learning mechanisms in simple systems. Several kinds of learning have been demonstrated (Jacobson, 1963, McConnell, 1965) in earthworms and marine polychaetes. The relatively simple ventral ganglia, composed of only a few hundred neurons, are nonetheless competent both to retain memories learned before decapitation and to learn after this operation, which removes the brain and subesophageal ganglion. As with other animals, a number of conditions, easily overlooked, can influence the apparent success in a learning test: earthworms may learn more quickly at night; some preparations may require intertrial intervals of only a few seconds; polychaetes differ from earthworms, and very probably differences may exist among families of polychaetes because their brains are highly divergent in advancement. In addition, very puzzling data exist on complexities in sensitization, and in interaction of two simultaneous habituation processes; Evans (1965) declares that proper controls for sensitization have not been run in many of the chief studies on polychaetes. Being soft-bodied, with simple unjointed appendages and a limited repertoire of behavior, annelids have attracted less physiological study than arthropods. Nevertheless, their availability, simplicity, phylogenetic position, learning capacity, and tolerance of mutilation suggest that they are well worth new attention.

Echinoderms

Starfish are said by Airapetianz and Sokolov (on the basis of recent work in the Pavlov Institute in Leningrad) to be able to learn a Y-maze (choosing arms on the basis of substrate texture), and to be classically conditioned by pairing light and food. Various kinds of persistent behavioral tendencies in righting and escape responses of asteroids and ophiuroids were described by Moore (1945) and others (see Warden, Jenkins and Warner, 1936). It is clear that we do not have a fair picture of the capacities and limitations of echinoderms or of many other lower groups in respect to types of learning.

Molluscs

Molluscs span an enormous range of nervous development (Bullock and Horridge, 1965) and behavioral complexity, from the lowly and the sessile forms (chitons, limpets, oysters, mussels, etc.) to the elaborate, active, highly visual and quickly teachable cephalopods (squid, cuttlefish, octopus). The latter are beyond our scope; little can be said relevant to our theme about plasticity in the lowest forms. But snails and their allies, especially the pulmonates, are intermediate and promise useful material. Warden, Jenkins, and Warner (1936) cite older experiments, some of which point to a very modest degree of habit formation and of classical conditioning in pulmonate snails.

Arthropods

Arthropods show several forms of learning (Warden, Jenkins, and Warner, 1936; Thorpe, 1963a; Thorpe and Davenport, 1965) and will not be considered here as intact organisms; but in Section III, below, simplified preparations are treated.

II. INTRODUCTION TO "SIMPLIFIED SYSTEMS"

"Simple systems" being investigated today, judging from the studies reported at this Work Session, include not just lower organisms but also isolated parts of organisms. Cohen described the goal of those working with such preparations as the finding of minimal physiological and anatomical systems that learn. Beyond this the use of such preparations opens up the possibility of chemical and other forms of analysis, and encourages studies of both the ontogenetic establishment of neural organization and of subsequent changes with experience. A further attraction for some workers is that efforts to determine the plastic and labile features of simple systems may give real insight into how the ordinary business of the nervous system is carried out and how innate behavior patterns work.

This chapter is concerned with those learning systems that an experimenter has artificially simplified by destroying unwanted parts of the nervous system, leaving only those parts considered relevant. There are two goals to this type of simplification which, while sometimes difficult to separate, should be clearly distinguished:

The first goal can be called the reduction of complexity. Learning situations, and indeed animals themselves, are exceedingly complex. Yet the number of variables relevant to learning can be reduced by simplifying the nervous system, so long as the remnant is still capable of learning. This type of simplification can contribute to conceptual clarity by depriving the system of many environmental factors that normally influence it through their action on certain sensory systems. Such simplification may also act, as in the studies on cockroach metathoracic ganglia (see pp. 126-8), by removing inhibitory mechanisms (in that instance, from the head ganglion) that ordinarily mask some of the simpler functions of the system under study. This first type of simplification, while resulting in fewer variables acting on the system, makes no claim for isolation of the system.

The second type of simplification has as its goal precisely the isolation of a system so that all inputs and outputs are observable and exhaustively described, even though mechanisms within the limits of some designated black box are still unknown. It is obvious that if a biologic system could be simplified so that all inputs and outputs are known, it could be more easily manipulated, and a number of mathematical and other formal models could then become applicable that are

only relevant to clearly and unambiguously isolated systems.

We intuitively conceive of there being a continuum of complexity from the intact human nervous system to in vitro systems. However, Crain's system (see p. 159), despite its being in vitro, is not as simplified as an isolated system since its inputs and outputs are unknown at the time of recording.

Another extremely important distinction to be drawn is that between studies of unit-cell "learning" (of the type reported by Buchwald et al. (1962,1965) in the spinal cord, Kamikawa et al. (1964) in the nonspecific thalamus, Olds and Olds (1961) in single cortical cells reinforced by brain stimulation, Yoshii and Ogura (1960) in reticular formation and Morrell (1961) in cortex) which belong to the first type of simplification, and Kandel's studies of the Aplysia abdominal ganglion cell, (see p. 141) which belong to the second, or isolation type. In all the unit-cell studies, an electrode (usually extracellular) records the spike discharge of a single cell, which can be treated as the effector system or response mechanism involved in the learning, and the nature of the inputs is not and perhaps cannot be determined.

In contrast to these studies, Kandel's ganglion cell has a limited number of output and input nerves. The transmission time from the input axon to the output axon can be used to determine the number of synapses involved. Although this method may introduce as yet unsuspected errors, it suggests the possibility of recording from a cell with no inputs other than those under the experimenter's direct control. It should be emphasized that the nature of the synapse is an open question, and that presynaptic inhibition remains a possibility.

The studies of the Adey, Olds, and Morrell type show the output behavior patterns of individual cells, and in this way give clues to processing in the nervous system. However, they do not really permit us to draw conclusions about the necessary conditions for these behavior changes in single cells. On the other hand, a study of the Kandel type, while it does provide an isolated system, may not permit generalization of conclusions to cells in other parts of the Aplysia nervous system or to other nervous systems, since the isolated cell may be atypical, and perhaps may not be particularly "intelligent." This inapplicability is particularly true if the experimental results are negative.

The authors know of no efforts to isolate nervous system

elements analogous to the Kandel model, using several neurons. The study of systems larger than one element would obviously be useful, provided that some monitoring of the functional behavior of each element were possible.

As Young stated in his 1952 Ferrier lecture, in general (but with some important exceptions) learning occurs in whole systems; one does not ordinarily look for learning in a giant axon or even in giant synapses with one-to-one conduction. Young stressed rightly, in Cohen's view, that interesting kinds of behavioral modifiability generally involve many neurons, often small and highly branched. Thus, in trying to look for a simple system that manifests learning, one is immediately caught in a paradox: If the system is sufficiently simplified anatomically, e.g., down to a giant axon or synapse, it may lose its behaviorally interesting characteristics, i.e., its plasticity or modifiability with use or disuse. One hopes to find a system that has sufficient units to produce interesting behavior but few enough to permit their interactions to be monitored. One of the various ways to approach this objective is to take an animal or part of an animal with known behavioral modifiability and to remove or ablate parts to see how far it can be simplified and still maintain that behavior. For example, the headless cockroach preparation of Horridge (see p. 122) has been used to determine how small a bit of the ventral nerve cord can show avoidance conditioning.

The technique of ablation brings with it logical problems, some of which came up for discussion at the Work Session. At first glance, "ablation logic" seems quite simple: If a selected behavior survives an ablation, one may say the region removed is not necessary for that behavior and that the remainder is adequate. However, when performance fails, one cannot say that the region removed is competent to learn, or that the remainder is incompetent. Closer approximation in actual cases often becomes quite difficult, due to secondary effects, traversing pathways, age-dependence, assumption of functions by the opposite side or other remaining structures, temporary shock-like effects, or interference with input, output, drive, alertness, and the like.

Nervous system preparations can be simplified by reducing the complexity of the input resulting from a stimulus, or by reducing the complexity of effector systems available for response, or by reducing the extent of the central tissue available to intervene. No one has tried to find the smallest sensory area required in the Horridge cockroach preparation

or the requirement, if any, for a specific modality of afferent input; but it seems likely to Hoyle from the experience to date that the input required is a small fraction of the sensory axons serving one leg.

The question of how small a part of the nervous system can learn is the basis for considerable controversy. The methodological problems for an informative answer are formidable. If one implies by the question that the given part must be surgically isolated from the rest of the CNS, as in the examples just discussed, then the isolation itself may so alter the natural capabilities -- by withdrawing tonic input, for example -- that actual localization may be missed. If the question implies, instead, that one must find the minimal number of units that can display learned activity, as recorded with microelectrodes, then the significance of results depends on one's distance from the final efferent path. There, the unit response will reflect both the learned act and the participation of ancillary structures. Concerning the electrophysiological interpretation of the question, a number of investigators studying electrical activity in single nerve cells or simple networks are finding changes that they consider to be a form of conditioning or learning.

The problem of defining learning is at the base of the question of minimal learning preparations; it, too, was given considerable attention at the Work Session (see Section VII, below).

Are there parts of nervous systems that cannot learn? The answer is thought to be yes. But there is too little established evidence for us to be able to say, for example, that the spinal cord or any other region that is not very specialized cannot learn. Despite the methodological problems involved, new work in this area would be worthwhile.

III. SIMPLIFIED SYSTEMS IN ARTHROPODS

As a reservoir of material for study, the Arthropoda, the largest and most diverse phylum, are of outstanding importance. They include the crustaceans, insects, arachnids, and their allies. These are mostly small animals and are regarded as having relatively few nerve cells (roughly in the range of 10^5-10^6, perhaps only 10^4 in minute insects). Their nervous system is generally subdivided into a string of bead-like ganglia, mostly concerned with the inputs and outputs of individual segments, each more or less equivalent to the others, hence offering ready-made simplification. Although these animals have a behavioral repertoire strikingly wider than the lower phyla, their behavior is predominantly stereotyped. Phenomena suggestive of learning seem to be a behavioral veneer, and are possibly rather simple in type.

Many arthropods noted for availability, amenability to experimentation, and for economic importance, such as the insects, have been the focus of past study. In the future, both insects and the more neglected arthropods such as crustaceans and arachnids are bound to yield new preparations of special value, such as Horridge's cockroach leg-lifting preparation. It would be a mistake to assume a priori that this preparation is representative of this large and diverse phylum, however.

Preparation of Horridge

In 1962 Horridge showed that a headless cockroach or locust, or even a part of the insect consisting of only one pair of legs (with its associated muscles, nerves, and ganglion), can exhibit behavioral changes satisfying the criteria of instrumental or operant conditioning (see Section VII). His original experimental design consisted of mounting the animal or preparation over water so that if the leg were allowed to extend it would touch the water, complete a circuit, and receive a mild electric shock. After some minutes of intermittently self-applied shocks, the leg remains flexed so that it does not touch the water. The period of flexion grows longer with repeated stimulation. To control for the possibility that this activity is due simply to shock repetition, Horridge arranged pairs of animals such that one, the so-called P animal, received a shock only when its leg was in a certain position, and the other, the random (R) or control animal, received the same shocks but, of course, in no consistent relation to its leg position. Only the P animal learns to flex its leg; the R animal does not, and may even become more prone

to make "mistakes" with time.

This simple experiment has attracted attention as one of the most promising bases for fresh work on the intimate correlates and essential conditions for learning. It is a simplified system, probably involving a small number of neurons -- a few score to a few hundred at most -- yet it is not so reduced as to appear irrelevant to many workers (Eisenstein and Cohen, 1965). It does act in a labile and seemingly complex way. Despite the promise of this preparation, however, improvements in methods of handling and selection are needed since at present predictability is poor, variance between specimens is great, and the useful life of the preparation is little more than a day. Eisenstein says that an isolated ganglion that has been trained rarely lasts more than 1-3 days, but if isolated and left alone, a ganglion may remain viable for as long as 10 days.*

At the Work Session, Hoyle reported on his own work, an elaboration of the studies begun by Horridge, on a simplified locust preparation. Hoyle utilizes what he calls an "electroneuropilogram (ENG)," a microelectrode recording of multiple units showing extracellular activity by which he can identify ganglionic sites associated with specific motor discharge. Electromyograms made from the leg are correlated with the ENG and also with electrical stimulation at another site. This correlative technique is difficult to use and Hoyle admits that experience is helpful in determining the appropriate part of the neuropile to examine for these correlated potentials. He showed an ENG associated with stimulation applied to giant

* Recently, Eisenstein (unpublished observations) has investigated the effects of various lesions in the CNS on shock avoidance learning using a modified Horridge preparation in which one prothoracic leg serves as the "P" leg and its contralateral mate as the "R" leg. With this preparation it is possible to record both "P" and "R" leg positions during training eliminating the need to run a test period and thus abolish the effects of training. The results to date with this preparation indicate that decapitated animals (i.e., ventral nerve cord or an isolated prothoracic ganglion) give larger differences between P and R legs than those with a head. If only the brain is removed, leaving the sub-esophageal and prothoracic ganglia joined, the P-R difference is abolished as both legs flex rapidly to the initial shocks and maintain their flexion. (Eisenstein)

axons of the cord; the efferent impulses to muscle are seen as vigorous bursts at first, but after several repetitions they fade and disappear. Hoyle explains this as a positive inhibition of ENG that may last for hours; he considers it to be habituation, although he admits more work is needed to clarify this.

Employing the locust, Hoyle has identified the muscle involved in the leg-raising experiments as the coxal adductor. When the whole animal is trained to flex its leg in order to avoid a shock, electromyograms recorded in this muscle show a small spontaneous discharge before learning that markedly increases after learning. Having identified the pertinent muscle, Hoyle then studied the headless locust, which is fixed in wax in such a way that no proprioception is produced by leg movement. Recording from this muscle, which has a single excitatory axon, he used a spontaneous fall in frequency of action potentials (judged by the investigator) as a signal to apply an electric shock to a sensory nerve (Hoyle, 1965). If the signal is judged properly and the shock is not too strong, conditioning occurs, as shown by an increased average frequency of action potentials.

In a more refined experiment, the tendon was cut and attached to a device recording the tension developed in the muscle. A sufficient change in tension was taken as a signal to apply stimulation. By either method, after a number of repetitions of an appropriate shock, suitably timed (apparently a certain level of skill is required), the average muscle activity due to the single excitatory axon increases to a new plateau. The fluctuating level of this tonic activity drops occasionally but there are increasingly longer intervals between drops. If the investigator raises the demand level for average activity, after a few shocks the mean activity rises so that again fewer shocks are required. In this way, an initial average frequency of 10 muscle action potentials per second can be raised to a level of 50 per second by progressively demanding higher levels, a few minutes to a level (Fig. 1). This can be reversed: with a high mean frequency, the shocks can be applied when momentary increases occur, whereupon the average frequency gradually decreases. By stages, the tonic activity of the muscle can be completely inhibited. Spontaneous inhibition sometimes causes temporary difficulty, but the mean frequency tends to return to the level at which the inhibition started. The effect of changing the average level of activity cannot be obtained simply by administering a given number of randomly timed shocks; in fact this is likely to make true instrumental conditioning more

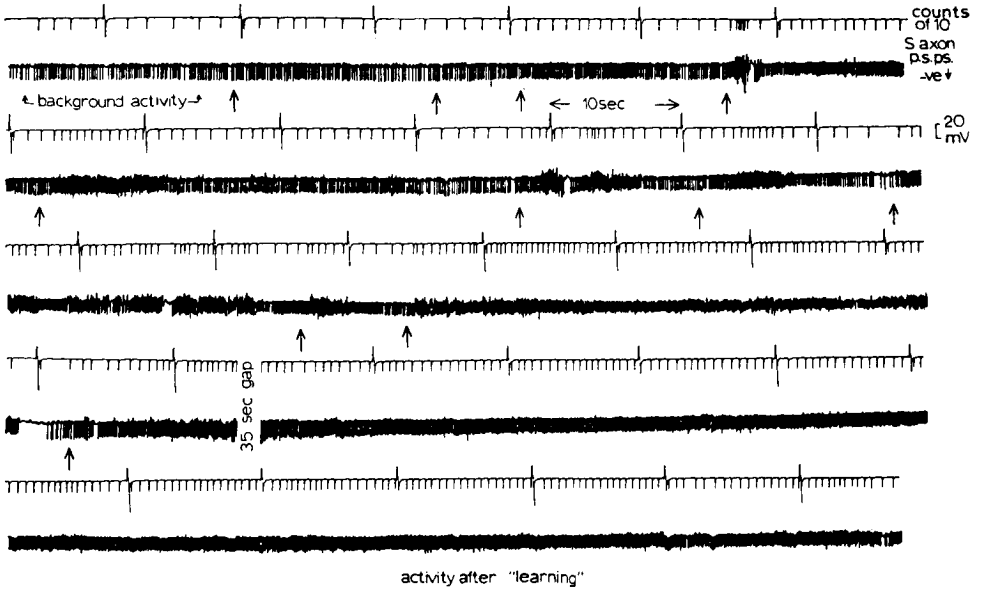

Figure 1. Maximum rate of "learning." Results of an experiment on the right metathoracic anterior coxal adductor of S. gregaria in which shocks (indicated by arrows) were applied to the leg at carefully judged moments, when an otherwise rising frequency started to fall appreciably compared with the previous 5-10 sec period. Electrical signals are intracellularly recorded junctional potentials; depolarization is downward. Background frequency at start was 6/sec. After 11 selectively timed shocks applied over a period of less than 4 min the background was raised to 20/sec. Upper trace: small downward pulses indicate every 10th pulse, larger artifacts coincide with print-out every 10 sec. The smaller potentials of opposite polarity are hyperpolarizing pulses, but they cause mechanical enhancement, not inhibition. [Hoyle, 1966].

difficult. The responses can be maintained at a given demand level by giving an occasional shock unless the demand level is very high, in which case there is more variation in frequency.

Hoyle went a stage further and took the muscle with its ganglion out of the animal to create an isolated preparation consisting of the metathoracic ganglion, the crural nerve, and the anterior coxal adductor muscle (Fig. 2). The source of stimulation and the force transducer were attached as before. It was found that "tweaking" the muscle with microforceps had the same effect as electric shock. Recordings from this preparation showed the same sort of activity as was seen in the previous headless animal preparation. Stimulation of the crural nerve when the frequency starts to decrease results in an increase which is maintained for longer periods of time.

The discharge of one motor unit does not seem to be correlated with activity in any other unit. That is, the learning experience of the anterior coxal adductor of the right metathoracic ganglion has almost no effect on the left metathoracic or either of the mesothoracic ganglia. Likewise, the presence of these other ganglia does not seem to interfere with the activity under observation. However, the experiment cannot be done well with the head attached because of an inhibitory influence and spontaneous bursts of excitation. Under present methods the isolated metathoracic preparation lives about 4 hours, after which spontaneous activity declines and then stops.

Hoyle believes his work indicates the possibility of a relatively long-term frequency change (many minutes) in the output from a single neuron induced by a simple sort of instrumental conditioning.

It is noteworthy that the instrumentally conditioned frequency can be either raised or lowered at will by a form of stimulation quite analogous to reinforcement. These results open up various avenues of new inquiry, and several obvious directions seem quite within the range of possible development, for example: reducing still further the sensory input, finding the most effective modality and pattern of input and of background state or stimuli, quantifying the minimal input, and experimenting with means to extend the life of the preparation.

Aranda and Luco (1966) found that in the isolated metathoracic segment which has developed the avoidance conditioning

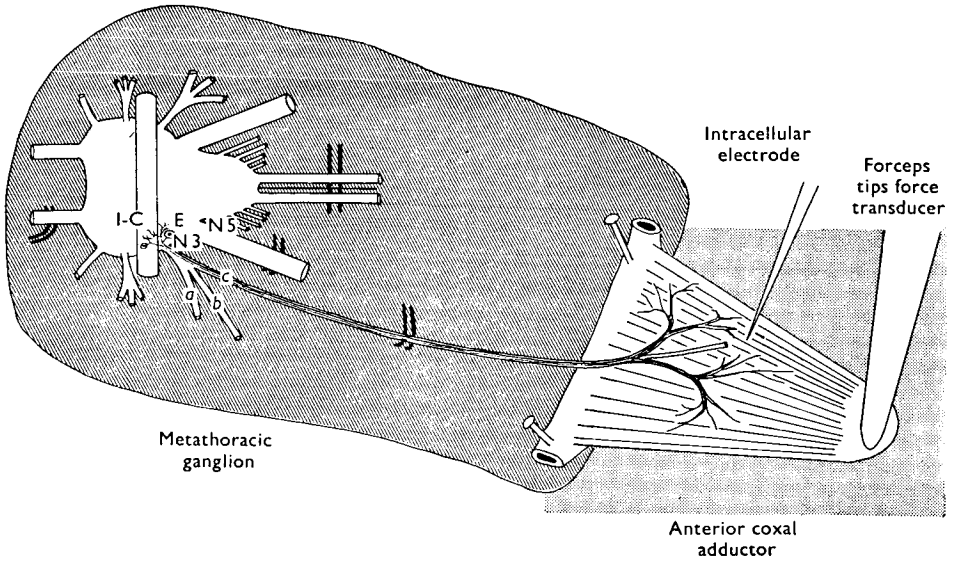

Figure 2. The isolated preparation (right, metathoracic anterior coxal adductor muscle). Principal positions of the stimulating electrodes used in eliciting responses are indicated. A single excitatory axon (E) and a single inhibitory-conditioning axon (I-C) innervate the muscle. [Hoyle, 1966].

posture, a nervous pathway with a synapse in that ganglion lowers its resistance to single stimuli to a preganglionic trunk (see Luco's preparation, below) and that spontaneous activity is higher in the trained side.

RNA Changes in Injured Neurons

Cohen reported on a special direction of new inquiry that grew out of work similar to that on learning in thoracic ganglia in roaches (Eisenstein and Cohen, 1965), namely, studies of RNA changes in the neuron soma following injury to the axon (Cohen and Jacklet, 1965). In the attempt to map arthropod ganglia (that is, identify a given cell body with a given peripheral axon in the leg nerve by use of the classic vertebrate chromatolysis technique), Cohen confirmed the findings of others (Hess, 1958; Wigglesworth, 1960) that arthropods, like most invertebrates, do not have Nissl bodies (sizeable clumps of ribosomal RNA layered on dense laminae of endoplasmic reticulum, that stain with basophilic dyes). Instead, arthropods have many ribosomes uniformly dispersed throughout the cytoplasm but very little endoplasmic reticulum. However, when Cohen examined 10μ sections of cockroach ganglia prepared within 12 hours after injury to a leg nerve and stained with a new pyronine-malachite green stain for RNA developed by Baker and Williams (1965), an aggregation and increase in RNA in the nerve cells was found -- in effect a reverse chromatolysis (see Fig. 3).

The nerve cell bodies of the arthropod ventral nerve cord are in a rind around the periphery of the ganglion surrounding the neuropile core. A transverse section through the metathoracic ganglion, for example, shows bilaterally matched pairs of cells; the ganglia are quite symmetrical. If the axon of one peripheral nerve is cut, its cell body can be compared to the contralateral mate with an intact axon. What is seen in the cell body of an injured axon, as early as 12 hours after injury, is a dense ring which appears around the nucleus. The control cell shows only a slight area of staining around its nucleus. These dense areas are accumulations of cytoplasmic RNA granules which appear as a solid band in the maximum state of response, about 2 days after injury.

Another interesting finding in an injured cell is a displacement of the nucleus from its normally central location in the cytoplasm to an eccentric location near the point where the axon emerges from the cell body. This displacement is not seen until about 3 weeks after injury.

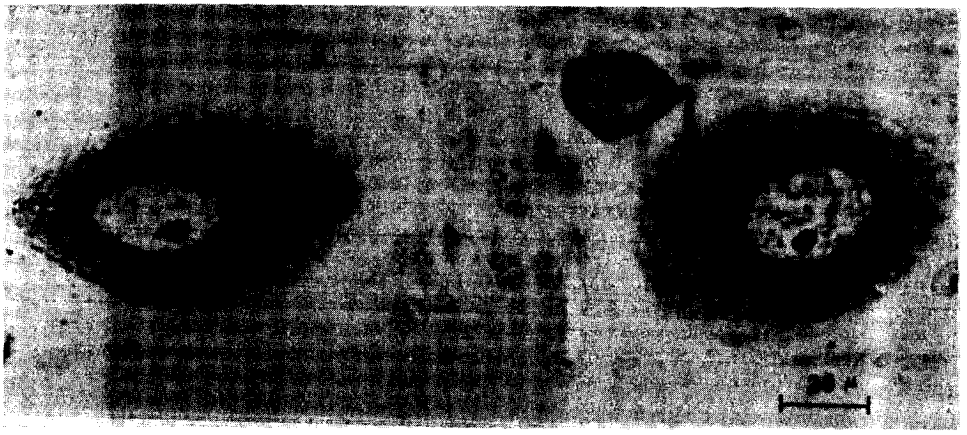

Figure 3. Matched central nerve cell bodies of a bilaterally symmetrical pair from the metathoracic ganglion of the cockroach Periplaneta americana. Stain is pyronine-malachite green to show cytoplasmic RNA. The axon of the cell on the right was cut 48 hours previous to fixing the ganglion; the axon of the matched cell on the left was left intact as a control. Note the dense ring of basophilic material in the perinuclear cytoplasm of the injured cell [Cohen and Jacklet, 1965].

In order to determine whether the RNA seen in the ring is newly produced or is previously existing cytoplasmic RNA drawn to a new locus in the cell, Cohen injected radioactive uridine around the ganglia of the animals at varying stages during the ring formation. Results of this work showed some of the RNA to be newly formed. Autoradiographs and pyronine staining of alternate thin sections revealed similar distributions of RNA in the cell, that is, in the nucleus, in the heavy ring outside the nucleus, and (very little) in the peripheral cytoplasm. There is a very short critical period during which label can be incorporated in the ring -- around 24 hours after injury plus or minus 2 or 3 hours. If the radioactive uridine is injected at other times, it is simply incorporated into the nucleus and then rather uniformly dispersed in the cytoplasm. Cohen is sure that the RNA does not move down the axon; rather, he believes the label is confined in the cell body.

When this material is examined with the electron microscope, it is seen to be essentially the same as vertebrate Nissl substance. An increase in endoplasmic reticulum heavily studded with ribosomes is visible in the area of the ring; this is practically absent from normal cockroaches. What is actually seen is a heavy preponderance of what are called "polyribosomes," rosettes of ribosomes which are indicative of protein synthesis. Within 2 weeks, the whole response disappears and the axon begins to regenerate. Cohen therefore interprets this RNA response as preparation for protein synthesis associated with regrowth of the injured axon.

These changes in injured cell bodies are so consistently and easily recognizable that Cohen is able to map the cells of the ganglia by cutting one nerve at a time and observing which cells show changes in response to injury. In an ongoing experimental series, nerves are injured at specific muscles in the expectation that association can be made between a specific nerve cell body and a specific muscle. So far about 53 motoneurons over 20μ in diameter have been identified and numbered. (See Fig. 4.) In observing these maps, the cellular symmetry in opposite halves of a ganglion is striking. Most cells send their axon out just one peripheral nerve and almost all are on the ipsilateral side. The cells for the muscle studied by Hoyle, the coxal adductor, so far cannot be identified individually but only as members of a small group of cells. It is not yet clear whether cutting a small branch of the axon will trigger the RNA ring reaction.

In relating this work to learning, Cohen described his

Figure 4. Cell map of the metathoracic ganglion of the cockroach Periplaneta americana. Reconstructed from 10μ serial sections. The solidly shaded cells send their axons out nerve trunks of the corresponding shading. The open shading shows the matching cells of the opposite side. Roman numerals indicate the number of peripheral nerve trunks. [Cohen and Jacklet, in press].

collaboration with Eisenstein, who has refined the preparation to a single ganglion and the two legs it innervates, one leg of which serves as the experimental or "P" leg and the other half as the control or "R" side. The results of RNA analysis on these ganglia are as yet inconclusive, but it seems that use alone produces rings.

Preparation of Luco

Another cockroach preparation, though approached quite differently, is that used by Luco (1964, et seq.). Cockroaches normally clean their antennae by pulling each one down to the mouth parts with the aid of an anterior leg. If the first pair of legs is amputated, the cockroach is unable to clean its antennae at first; but after 8 to 10 days it learns to do so (Fig. 5). Luco has shown that while the roach apparently learns to use its middle legs to manipulate its antennae, this is not the actual learned act, since a cockroach with its front legs removed, if fastened with its back down, is immediately able to clean its antennae with its middle legs. Evidently the animal actually learns to stand on three legs placed so that the center of gravity is inside the tripod, freeing the fourth leg for cleaning. Thus, a postural act is learned, and not a manipulative skill.

If the forelegs of a fairly young cockroach are cut, they will grow back. When this occurs, the animal that has learned to clean with its middle legs will resume the normal practice of cleaning with its forelegs. If these regrown forelegs are then removed (even up to 20 days after the use of forelegs has been resumed), the animal immediately is able to use the middle legs without the period of learning that was required after the first amputation. This seems to be evidence for a retention of this learned maneuver.

Roeder reported that the attempts to duplicate these results using a different species, Periplaneta americana failed; the roaches never learned to use their middle legs for cleaning after foreleg amputation. Luco thinks this may be a species difference and cites observations (personal communication) by B. H. Smith in F. O. Schmitt's M.I.T. laboratory, to the effect that Periplaneta americana from Boston are able to bring the antennae to the palps for cleaning by using the antennae muscles without the help of the legs.

Luco reported an electrophysiological correlate of the cockroach learning. Stimulating a preganglionic connective and recording from a branch of a peripheral nerve containing

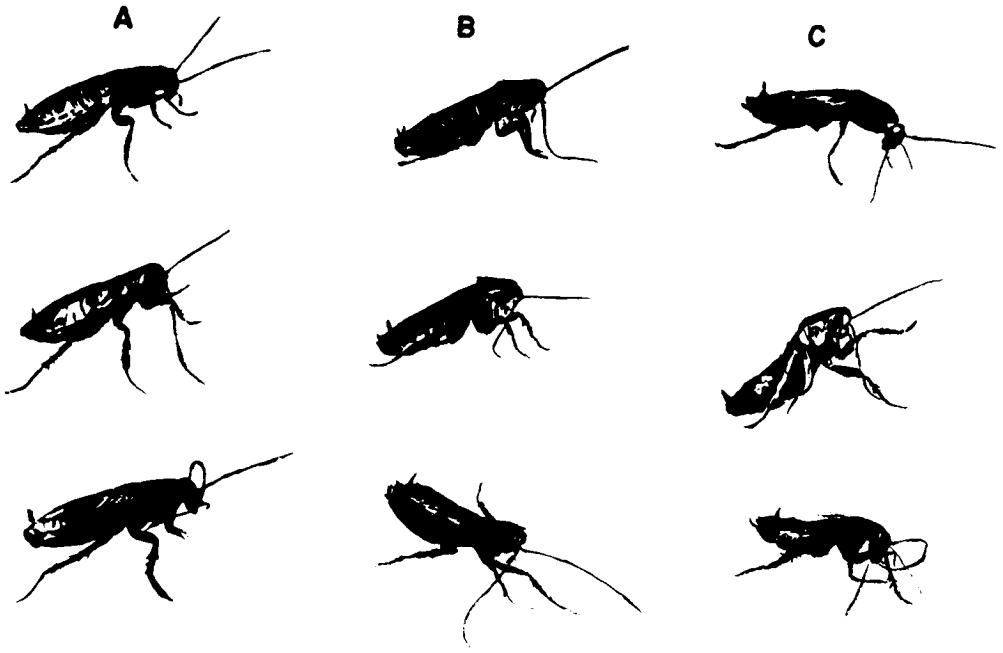

Figure 5. The performance of cleaning the antenna. A: normal cockroach: the antenna is brought to palps with one foreleg. B: 3 days after removal of the two forelegs: unsuccessful efforts to catch the antenna with one middle leg. C: 20 days after the same operation: antenna is pulled down with one middle leg [Luco, 1964].

a small number of motor axons, he found that 10% of normal roaches show a rather labile efferent response with an initial deflection at about 4 msec latency. A similar response is given by preparations made from roaches whose forelegs have been amputated within the last few days, but not the 8 or 10 days needed to learn the compensatory skill. But nearly all preparations respond if made from roaches that have had 8 or 10 days to recover and to show the learned skill; the initial deflection is earlier, at about 2 msec, and is much less labile (Fig. 6). Luco believes that this consistent difference in electrophysiological properties is relevant to learning (Fig.7), but he has no definite proof of this. The electrical response observed in cockroaches fastened on their backs for 10 days is not modified by cutting the forelegs at the beginning of that period (Davidovich et al., 1966). After regeneration of the forelegs in free animals and resumption of the normal method of cleaning, 100% of the cockroaches present a labile response, i.e., there is partial return to normal. (In only 10% of normal cockroaches is a labile response observed.) In addition, after forelegs were severed in cockroaches with regenerated forelegs, the response appeared in only 3-4 days instead of the 8-10 days needed by the normal roaches. Therefore, Luco concludes the savings mentioned above in such animals are stored in this transmission mechanism for at least 20 days during which no reinforcement is present.

Chorover cited Luco's experiment regarding the antenna-cleaning response of cockroaches with amputated forelegs as a good example of the general dictum that close analysis is needed to determine the specific response made to a given aspect of the stimulus. Chorover suggested that the problem of properly relating stimulus and response has implications for electrophysiological studies as well. It cannot be immediately assumed that a given electrophysiological property is due to the aspect of the situation that may have caught our attention. For example, Schiller and Chorover (1966a) have found that amplitude and latency of visual evoked responses correlate with the physical intensity of the stimulus and not with the perceived brightness. Whatever the definition of learning, one always infers and never directly observes that learning has occurred. The inference is made on the basis of behavior; in that sense learning is a hypothetical construct that we build to relate the input and output characteristics of a behaving organism. But it is not always apparent how to distinguish between learning and a more general change in performance. The general and specific results of any treatment, from the crude intervention of amputation to the subtle effects of drugs, may separately alter an animal's learning and its ability to perform.

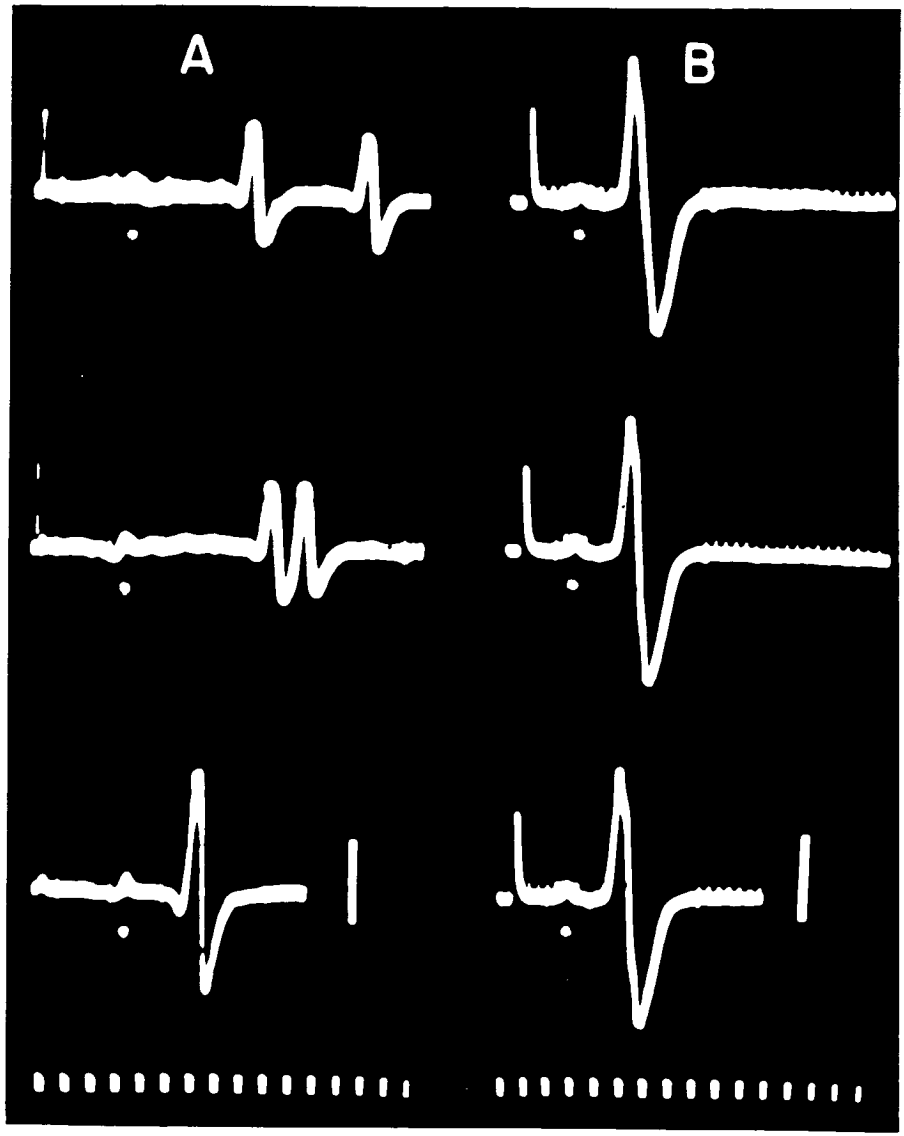

Figure 6. Delay characteristics of responses from a normal insect and from a roach with its forelegs amputated twelve days before. Stimulating and recording electrodes as in Fig. 5. A: normal cockroach; B: operated cockroach. At A, two single units are active. At B, three units (as demonstrated during fatigue) are responding at the same time. Calibration: 200μV. Time: msec [Luco, 1964].

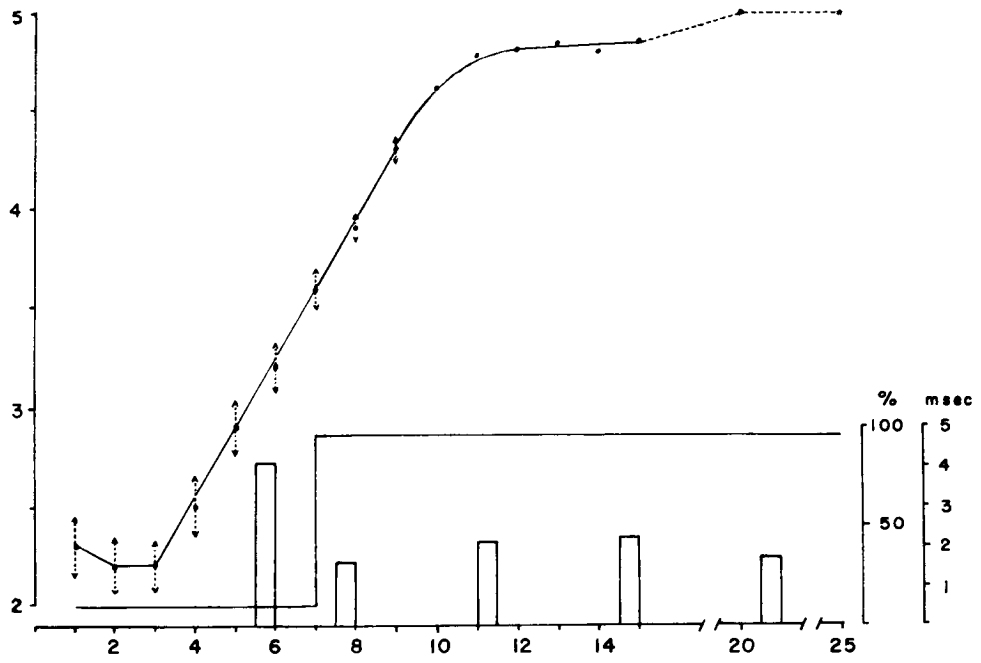

Figure 7. Time course of behavior and electrophysiological phenomena in the cockroach without the two forelegs. The curve with the standard errors represents the progressive behavioral improvement. Abscissae, days after removal of the two forelegs. Ordinate (at left), conventional scale to measure the ability to perform. The open columns represent the ganglionic delay measured in msc (ordinate at right). A lower curve shows percentage of cases presenting output-signals (ordinate at right) [Luco, 1964].

McCleary agreed with these points and added that the cockroach experiment is a good learning experiment because it deals with a nearly intact animal, it does not drastically interfere with the nervous system, and it takes advantage of soemthing the animal normally does. This preparation should therefore be exploited further.

Another type of preparation may be mentioned because it deserves new study. Schöne (1965) has shown the quantitative dependence of postural responses of crustaceans upon the summation of amount of input from mechanoreceptors such as the statocysts. This input depends on the weight of the statoliths which are renewed at each molt, gradually increase with size of the animal, and probably fluctuate from molt to molt enough to affect the input. A central adjustment of the transfer functions or calibrations may be reasonably suggested. The ability to do this is demonstrated in Schöne's work on recovery from the initial disorientation due to injury of the statocyst nerve of one side. Perhaps widespread in arthropods is a relearning by operant conditioning after each molt of the weighting functions assigned to many receptors. All sensory hairs and other exoskeletal organs are shed and renewed, doubtless with some degree of mechanical alteration. As a first-order experiment, at least, it would be interesting to see whether postural reflexes are restored if a statolith deficit or excess occurs (by molting or experimental intervention) and the animal is deprived of relearning experience (for example, by being suspended).

IV. SIMPLIFIED SYSTEMS IN MOLLUSCS

Among the many possible preparations in the phylum Mollusca, only one has received much attention recently, namely the parietovisceral ganglion of the marine opisthobranchiate gastropod Aplysia ("sea hare"). Although extensive work on learning has been done in cephalopods, it is excluded here on grounds of complexity.

The paired ganglia of the gastropod nervous system typically include: a pair of cerebral or supraesophageal ganglia, a pair of buccal ganglia, a pair of pleurals receiving connectives from the cerebral and pedal ganglia and the visceral loop, a pair of pedal ganglia, a supraintestinal ganglion and subintestinal ganglion, a pair of parietal ganglia, and a visceral ganglion. These are more or less concentrated in different species by shortening of their connectives to various degrees. In Aplysia all are close together except the abdominal or parieto-visceral ganglion, which lies far from the circumpharyngeal ring, well-surrounded by connective tissue and housing about 1000 nerve cells of the approximately 10,000 in the whole central nervous system.

Biological Clock Studies of Strumwasser

One of the modern Aplysia studies that has a bearing on our theme is Strumwasser's. In isolated Aplysia abdominal ganglia kept alive for several days in seawater, he has studied neuronal clocks that can store information as to the time of the last resetting of a circadian timekeeper. "Store" in this case is not a static memory but a dynamic process of keeping track of the fraction of a cycle elapsed since the last setting or its circadian "anniversary." A certain identifiable ganglionic cell has been shown to exhibit consistently a circadian rhythm of spontaneous firing: there is a peak and a trough of activity during each 24-hour period. Regardless of when the animal is killed and the ganglion removed, these will be found at a predictable phase relative to the animal's prior light-dark regime. This cell can be entrained by a shifted light-dark regime; it will continue to wax and wane in this rhythm as long as the isolated ganglion survives. In Strumwasser's experiment, a group of animals is kept under one regime; each day at various times a preparation is made from one of them, each preparation surviving about 2 days. The cells are impaled and the microelectrodes maintained intracellularly for long periods of observation of electrical activity.

In the standard procedure the animals are kept in constant light for one week before the conditioning or resetting regime. During this time the activity peaks are broad and low, forming roughly a sine wave of 12 hours of depressed and 12 hours of enhanced activity. The imposed dark-light cycle (for about 2 days) produces changes both in form and in phase. The form change is from a broad peak to a sharp peak of 2 to 3 hours. The phasing or synchronization change causes about 85% of the cells in the isolated ganglion to peak when the light would have come on, while 5% peak when it would have gone off, and 10% peak at both times.

When the activity of the cells is studied over a long time, the oscillation is seen to have a 2-week period, which lags slightly behind the time of high tide at the locality where the specimens were collected. There is a daily 10-minute deviation, with confidence limits of about 2 minutes for the 95% level.

When the light schedule is shifted 4 hours earlier, 4 days are required before the animals are synchronized with the new schedule. But even after one cycle of the new dark-light regime there is an indication that something has changed in the behavior of the nerve cell. To Strumwasser this is an important example at the cellular level of a presumably genetically prepared, predisposed behavioral program that can be modified by experience and can store a record of this experience in the reset phase.

One possible explanation of this phenomenon was that the cell counts the spikes in its output over a long time period and initiates the depression after a certain number of impulses, in this way generating a clock mechanism. However, this explanation was ruled out by hyperpolarizing the cell so that the pacemaker could not cause spikes; the expected rhythm nonetheless came through in pacemaker potentials.

The possibility that other pacemaker cells act as internal inputs to the cell and possibly determine the rhythm was considered, but inputs from other cells are visible as synaptic potentials and none of these systematically changes in frequency in a circadian rhythm.

Several procedures can alter the peak's appearance. By applying temperature pulses of about 10° rise during the projected dark period, there is an acceleration of the appearance of the peak. When the temperature pulses are stopped, there is a higher latent peak. This is interpreted as being

displaced activity; Strumwasser thinks he has accelerated the "message," either by synthesis or release mechanisms.

The activity peak can also be chemically manipulated by injecting materials into the cell. Potassium sulfate has no effect on the frequency of spike output. But an equivalent amount of injected chloride produces a flattening of the post-burst hyperpolarization; the cell stops bursting for 15 minutes, then returns with an overshooting response of post-burst hyperpolarization that decreases as bursts begin. This suggests that chloride is important in generating the hyperpolarization between bursts.

Actinomycin-D is well known to bind with DNA and prevent the formation of messenger RNA. When actinomycin-D is injected in about the same amount as potassium sulfate during the dark period, it seems to release immediately the peak that was to have occurred at the dark-light transition. In order to maximize the amount of time available to analyze the results of actinomycin injection, Strumwasser took an intact animal conditioned for 10 days with a light-dark cycle, removed the ganglion after the time of a normal peak, impaled the cell, and within one-half hour injected actinomycin. A weak effect, releasing a burst was seen. However, on the second day the peak was synchronized with the time of the actinomycin injection instead of occurring at the previously normal time.

It is Strumwasser's tentative hypothesis that a message is produced, presumably by the nucleus and possibly via messenger RNA. The product ultimately produced by the enzymes thus synthesized, then depolarizes the cell either by interacting with the inside of the membrane or by traversing the membrane and interacting with the outer surface.

Puromycin and ribonuclease do not shift the phasing (they only temporarily block the expression of bursting), but they do depolarize the cell for about 12 hours. Schmitt considered this effect on the cell to be of great significance in view of the growing evidence that the electrogenic molecules which gate ion passage through the membrane are protein or conjugated protein capable of fast conformation change (Schmitt, 1967). Agranoff wondered whether the actinomycin ultimately destroys the cell. In many in vivo applications, the organism dies as a result of actinomycin-D treatment.

Strumwasser has done some analysis of the DNA content of the cells and finds about 50 thousand times more DNA than

has been found in the cells of mammals.

Finally, he reported experiments to determine the relative amounts of RNA in cells using acridine orange and DNAase with ultraviolet irradiation. He hopes this technique will show whether during the cell's activity cycle the uridine moves out of the nucleus at some time related to the activity peak.

Conditioning Studies of Kandel

Kandel described work on Aplysia with Tauc in Paris, continued more recently in collaboration with Frazier and Waziri. The general plan is to see whether a series of stimulus-patterning sequences, derived from conditioning paradigms and useful in generating learning and complex behavior in intact higher animals, might be fruitfully applied to the cells of the isolated ganglion. He has studied both classical and instrumental conditioning.

The classical conditioning paradigm consists of taking two stimuli of differing efficacy -- an "unconditioned" stimulus (US) that is effective in triggering a response, and a "conditioned" stimulus (CS) that is not initially effective. By pairing these two stimuli repeatedly in the order CS-US, one finds an increase in efficacy of the initially ineffective CS, and thereby produces classical conditioning. Applying this paradigm to the isolated ganglion, Kandel records from a single cell and stimulates two different nerves leading to the ganglion, varying the stimulus intensity and selecting two purely excitatory inputs. The efficacy of the two pathways is controlled so that one (which he calls the "test stimulus") produces only a small synaptic potential; this is the analog of the conditioned stimulus, CS. The stimulus to the second pathway (which he calls the "priming stimulus", although in the classical conditioning paradigm it follows the "test" stimulus) is more effective and produces a larger synaptic potential and a train of spikes; it is the analog of the unconditioned stimulus, US (Fig. 8). The question to be asked is: What is the response obtained from stimulating the first pathway alone (the less effective input) after it has been followed repeatedly and intermittently by the second input?

For most of the cells observed, the effect of the weaker input alone is not significantly enhanced after being repeatedly followed by the stronger input, using different relative efficacies. However, some effect of input pairing was found

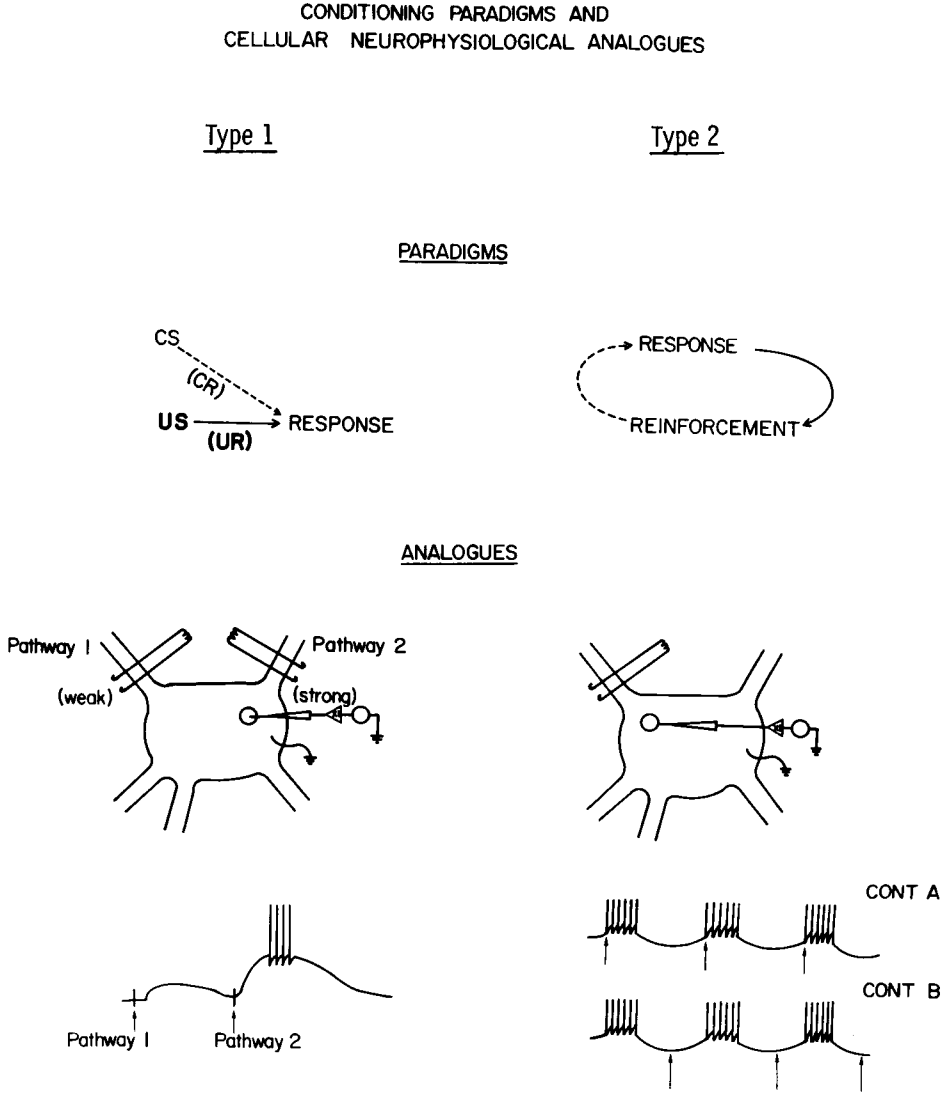

Figure 8. The relationship of the stimulus sequences of the type 1 and type 2 conditioning paradigms to the cellular analogs. The left part of this figure describes the type 1 procedure, the right part the type 2 procedure. The top section of the figure illustrates a conventional statement on the paradigm. The middle section illustrates how these statements can be applied to the isolated ganglion using electrical stimuli instead of behavioral ones. The bottom section indicates the intracellular recorded indices that have been used as the response in these cellular analogs. [Kandel].

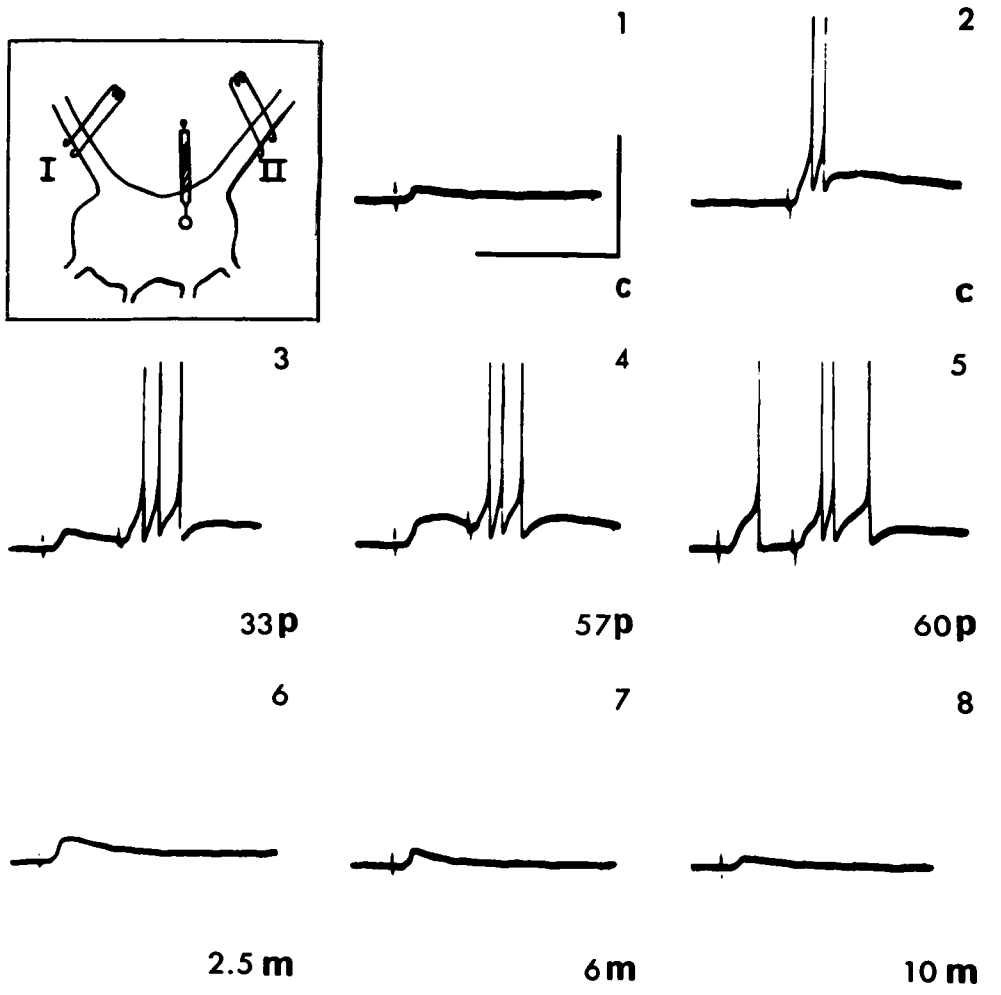

Figure 9. Heterosynaptic facilitation. The experimental arrangement is indicated in the inset. The test stimulus was a single shock to the left connective, the priming stimulus a single shock of stronger intensity to the right connective. Parts 1 and 2 illustrate the control response to the test and priming stimulus respectively before pairing. Parts 3 to 5 show changes in the test PSP following 5.5 min, 9.5 min and 10 min of pairing (33, 57, and 60 pairing trials respectively). Note that the augmentation in the test PSP has also produced a slight facilitation in the response to the priming stimulus. Parts 6 to 8 illustrate the decline in the test PSP in the 10 minutes following pairing. The action potentials have been retouched. [Kandel].

in a small number (15) of unidentified cells located in the right upper quadrant of the ganglion and in the giant cell also located in this quadrant. Figure 9 is an example of this early work in which an unusually long period of pairing was used and only a single strong stimulus served as the "priming stimulus" (US). Trial pairs were given every 10 seconds, with the weaker stimulus preceding the stronger by about 200-500 msec. After about 5½ minutes of pairing (33 trials), a slight increase was seen in the first synaptic potential. After about 9½ minutes, in addition to the larger synaptic potential, there was also a local response; and after 10 minutes, spikes were triggered. When the pairing was stopped, after about 20 minutes, the synaptic potential slowly began to decrease; it returned to its control value in another 10 minutes. Since the increased response to the weaker, first stimulus is attributable to the antecedent activity of previous trials, it can be called "facilitation," whether or not it is specific to pairing; since the two stimuli are given to different preganglionic nerves, it is called "heterosynaptic."

It was possible to obtain more effective facilitation with fewer pairing trials by using a train of stimuli as the stronger "priming" stimulus rather than a single shock. Since the percentage increase in the synaptic potential was the significant dependent variable, and not the presence or absence of spikes, precautions were also taken to prevent spiking. This permitted an accurate measure of changes in excitatory postsynaptic potential (EPSP) amplitude throughout a wide range. With these modifications in technique, it was possible to observe among the unidentified cells that a small test synaptic potential (analog of conditioned response) increased an average of two-fold in amplitude in 16 to 30 pairing trials, then returned to control value within an average of 9 minutes after pairing. In three cases control experiments were run with variations in pairings and in these cells the effect was seen to be due to the concomitant pairing of the two inputs and did not occur if the two inputs were not paired. In three additional cases a specificity to paired input was demonstrated. However, "backward conditioning" (US then CS) and random timing of US and CS were not tried.

In the "giant" cell, one of the readily identifiable cells of the ganglion, a much more detailed study of a form of heterosynaptic facilitation was undertaken. Facilitation in the giant cell differed in several ways from that in the unidentified cells: 1) optimal facilitation required fewer pairing trials (3-9) for maximum effectiveness; 2) the facilitation was larger (100% - 700%) and lasted longer (up to 40

minutes) than that seen among the unidentified cells; 3) it was not specific to pairing or to paired input. The sensitization controls show that almost comparable facilitation is obtained without sending in the weaker input each time. Intermittent reinforcement may lengthen the time needed for extinction in the giant cell. Heterosynaptic facilitation in the giant cell could also be produced by a natural stimulus (i.e., stroking the skin, which causes a brisk discharge in the giant cell) following a weak electrical stimulus to one of the connectives.

In view of the large size and ready accessibility of the giant cell, Kandel was able to look into the cellular mechanism underlying the facilitation found in this cell. He found no change in the membrane resistance of the cell. He suggested that the change occurs in the input to the cell, either by an increase in the excitatory drive or by a decrease in inhibition. Disinhibition was excluded by poisoning the ganglion with curare without destroying "facilitation." (Tauc and Gerschenfeld (1962) have shown that inhibition in the ganglion is almost entirely cholinergic.) It would seem, then, that there is an increase in the excitatory drive to the cell which could result from either more units coming into the weaker (CS) pathway or from an increase in the efficacy of the same units. Kandel noted that careful comparison of postsynaptic potentials from different nerves before and after facilitation showed that notches on the rising phase of complex excitatory postsynaptic potentials are facilitated. He suggested that this may be due to a presynaptic facilitation; that is, an interaction between the US pathway and the CS pathway. The US pathway would not only form a synapse on the postneuron (from which he records), but would also send a branch that makes functional contact with the presynaptic terminals of the CS input and controls the amount of transmitter substance released per impulse.

To test this hypothesis Kandel developed criteria for monosynaptic inputs to a giant cell and consistent with this hypothesis showed that this type of facilitation could occur in an elementary monosynaptic EPSP. The alternative mechanism of heterosynaptic post-tetanic potentiation, although less likely, could not be completely excluded.

The proposed presynaptic mechanism of heterosynaptic facilitation is only applicable to the nonspecific form of facilitation. The mechanism for the specific form is still undetermined but a nonspecific presynaptic facilitation could exhibit specificity if the axon terminals in some of the

unidentified cells would undergo facilitation only when they themselves had been invaded by an action potential some several hundred milliseconds before the impingement of the "priming" stimulus. Specificity could also be conferred by means of appropriate convergence and divergence in a complex neural circuitry.

Turning to the study of the instrumental conditioning paradigm, Kandel described experiments in which he recorded from an identifiable cell that bursts in a fairly regular manner. In accordance with the definition of this type of conditioning, stimulation is applied contingent upon some activity that the cell manifests of its own accord from time to time. In the present case, two types of contingency could be specified, according to the timing of the stimuli: contingency A, in which the stimulus arrives at the beginning of the naturally occurring burst and causes an earlier appearance of the next burst; and contingency B, in which the stimulus is applied during the quiet period and causes a lengthening of that period. Burst generation in these cells appears to be endogenous, but this endogenous rhythm can be modulated by neural input. Kandel points to the contingency-specific effects of intercollated stimulation as suggestive of an operant conditioning analogy. In some cells there is a buildup of effect over a number of stimulations and in some there is a persistence of the altered interburst interval for many cycles (up to 30 minutes) after the last stimulation. In addition to contingency-specific effects, which were obtained with weak stimuli, it was readily possible to produce prolonged nonspecific effects (lasting 10 to 20 minutes) with strong noncontingent stimuli.

In discussion, the question arose whether the contingency-A experiments constituted instrumental conditioning. Kandel explained that the burst is the contingency and the reinforcement is applied as soon as the burst occurs. Chorover likened contingency A to positively reinforced operant responding, in which the frequency of response increases (i.e., interresponse interval decreases) with reinforcement. Contingency B appears comparable to a "punishment" situation in which response probability decreases (or inter-response interval increases) when the response is followed by an aversive stimulus. The trouble with the latter analogy is that to parallel the behavioral findings, the stimulation should have a comparable (or even greater) suppressing effect when it is administered during (rather than after) the burst response. In the absence of such findings, a simpler, proactive interference (inhibition?) effect seems to account adequately for observations made under contingency B.

Habituation Studies of Tauc

Tauc reported on work with Bruner (1964,1965,1966) involving habituation in Aplysia. When drops of water are allowed to fall on its head, the animal contracts. However, if this is repeated several times at 30-second intervals, the response becomes less and less. If the animal is allowed to rest for 10 minutes, dishabituation occurs, i.e., the initial response is partially recovered, although it subsequently seems to habituate faster. Dishabituation can also be obtained by applying some external stimulus, such as scratching the skin elsewhere. Thus, true habituation to this kind of stimulus does occur in Aplysia.

In order to study this phenomenon electrophysiologically, Tauc and his collaborators use a preparation that includes the head and upper ganglia; they record with a microelectrode in the left giant cell. With the first drop of water on the head, there is a large postsynaptic potential; this decreases with subsequent drops until, after the 15th, the response is very small though the activity recorded in the nerves from the head has not decreased. (See Fig. 10.) After 15 minutes of rest, the initial response is recovered; when drops are resumed, the response decreases faster. Recovery can also be obtained by introducing external stimulation, such as scratching the head or stimulating some of the other nerves. Tauc suggests that this dishabituation is a central phenomenon. The giant cell is not involved in the reflex loop that produces body contraction. Therefore, he reasoned, if this is a central phenomenon the head is not needed and stimulation of the preganglionic nerve should be sufficient to produce habituation. This was demonstrated to be true. With the drops on the head, there is a compound potential. In the case of stimulation of the nerve, there is the possibility of activating only one interneuron and a unitary postsynaptic potential is obtained; recovery is slow. Stimulation of other nerves that synapse on the same cell, though not coming from the head, causes the same phenomenon and the recovery from this is even longer.

When, instead of a single stimulus, one applies a train of shocks to the same pathway producing the monosynaptic response, clear habituation appears. During successive trains of stimuli separated by periods of rest to permit recovery, habituation occurs faster and the recovery needed is longer. After a few trains of stimuli more than one hour is necessary to come back to the initial amplitude of the responses. However, if one stimulates the cell directly, through the

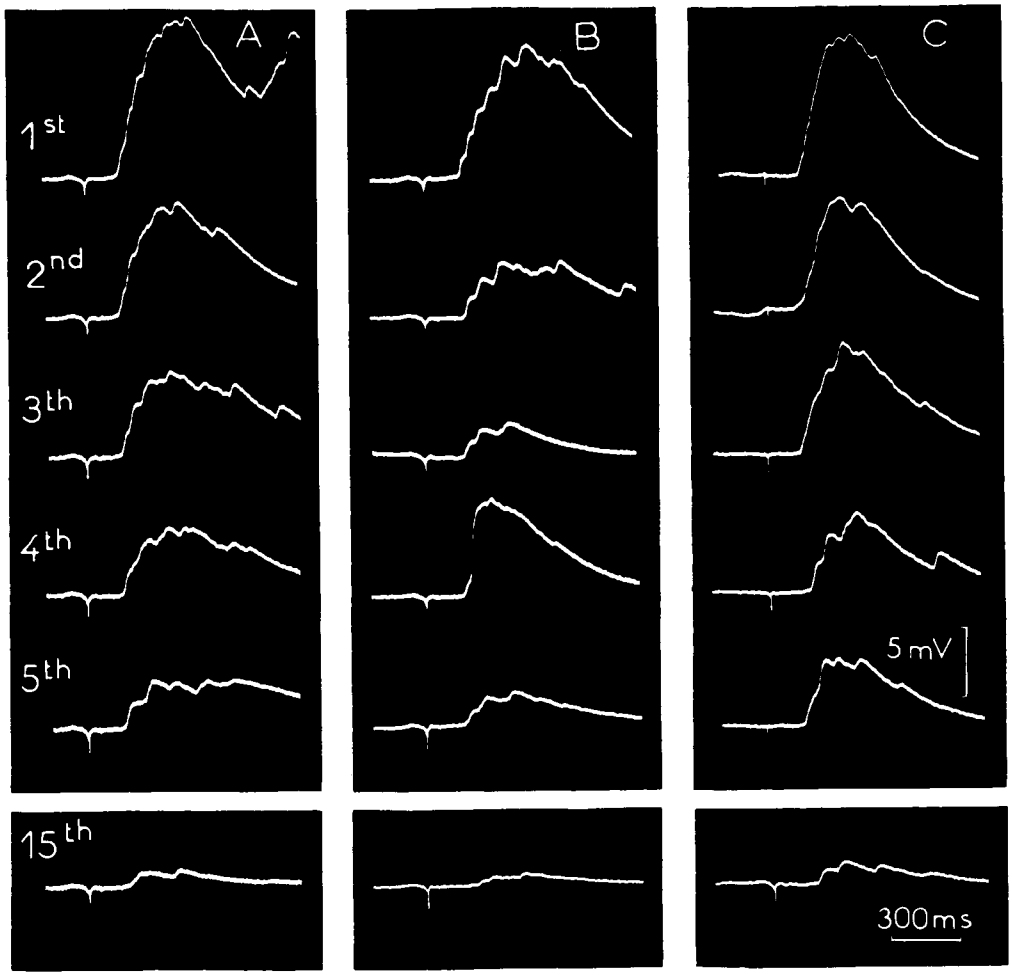

Figure 10. Modifications of compound EPSP during habituation and dishabituation produced by drops applied at 10-sec intervals on the head near the left anterior tentacle of Aplysia. A: First series of fifteen stimuli. B: Second series of stimuli showing restoration of the EPSP after 10-min rest. C: Continuation of series B without break in stimulation frequency but following a 2-sec repetitive stimulation (5 percent) of the left posterior pedial nerve. Note the restoration of the initial amplitude of the EPSP. [Bruner and Tauc, 1966].

intracellular electrode, habituation is not produced; apparently a synaptic pathway is needed.

Tauc uses the term "coefficient of habituation" to describe the change in response between the first and second or third stimuli. Synapses that have a high coefficient of habituation are extremely sensitive to magnesium, less to calcium. The contrary is true for synapses which do not habituate (Tauc, Epstein, and Mallart, 1965). Since both magnesium and calcium ions can change the quantity of transmitter released at the synapse, Tauc considers this an indication that the mechanism of transmitter release can be involved in habituation.

Segundo reported on work with cats which is relevant to that of Tauc. He has studied neurons in the mesencephalic and bulbar reticular nuclei. Recording activity extracellularly one observes that when a certain stimulation is repeated about every 2 or 3 seconds, there is a gradual decay or "attenuation" of the response. If a strong stimulus is interjected, the response returns. When recording intracellularly, one observes a decay in the size of the postsynaptic potential (PSP), and there are no signs of counterbalancing PSP's as membrane potential shifts, or spike excitability changes. Segundo feels that this work supports Tauc's interpretation that repeated activity is followed by some protracted change (e.g., depletion of transmitter) at the presynaptic terminal level. McCleary offered an important criticism by asking how the dishabituating stimulus can cause a return of the response if some aspect of the presynaptic terminal function had declined. Segundo suggested that since the dishabituating stimulus must be strong, it may produce an independent excitability change in the nervous system. Tauc thinks there may be a change in the properties of the presynaptic fiber, perhaps in the polarization induced by the heterosynaptic impulse, as in the several cases already known of presynaptic synapses, or a change in the mechanism more directly involved in transmitter release.

One feels that the search for plastic properties in paucineuron preparations, meaning the whole gamut of changing properties including those suggestive of learning, is not only important and fruitful as these pioneering studies show, but still has its main future before it. Deliberate effort to elicit plastic properties, using diverse paradigms, should be focused on a wide assortment of cells and animals. At this level, higher than most of our experience with neuronal physiology, it is not to be assumed that we have an adequate sample

or a balanced perspective on all the conditions for, types of, properties of, or even the best examples of plastic behavior in nerve cells, with only these and the other few pioneering studies.

V. RELATIVELY SIMPLE SYSTEMS IN VERTEBRATES

There is a common identification of "simple" with "invertebrate" but this identification is without zoological, let alone logical or behavioral basis. "Simple" in our context means relative to the learning achievements of man and his closer allies. Some extremely interesting work has been done on lower levels of the vertebrate nervous system and lower classes of vertebrates. No comprehensive review is undertaken here, but certain instances will be mentioned in these two spheres.

Persistent Plastic Changes in the Spinal Cord

Certain changes in connections following environmental impact have been described (Bullock and Horridge, 1965) that have the nature of regulatory readjustments of reflex pathways. For example, man and other primates can recover considerably from an initial deficit after many types of lesions to either the peripheral or central nervous system. These include nerves cross-sutured into the wrong peripheral field, and gross lesions in cerebellum and cerebral cortex, even in specific motor areas. By no means can all central recovery be attributed to recovery from shock. Younger animals show less shock and less permanent deficit from the same lesion than adults. In man this recovery is often called "relearning" and is markedly increased with high motivation. Adult lower forms, including rats, frogs, and fish, recover less easily. Rabbits, however, are said to show significant readjustment after the crossing of flexor and extensor nerves or tendons, though recovery requires 8 to 12 months (Shamarina, 1958). At least as low as crustaceans there is found complete recovery in a few days from the severe initial disorientation caused by unilateral damage to the nerves from the equilibrium receptors (Schöne, 1954) and very likely there is a central adjustment probably equivalent to operant conditioning after each molt to compensate for differences in the weight of the new statoliths compared to the old ones. In very young stages, even amphibians can apparently rearrange central connections after cross-union of flexor and extensor nerves to give normal movements. A supernumerary grafted limb innervated by nerves not normally supplying limb muscles gives coordinated movements in phase with the normal limbs, and even cutaneous reflexes like those elicited by stimulating corresponding points on normal limbs. This ability declines as ontogenesis proceeds (Weiss, 1936, 1950,1965).

While these phenomena show a remarkable adaptive

plasticity and compensatory self-regulation, they are hardly ever treated as examples of learning. Nevertheless, they illustrate the use, by higher animals especially, of lability presumed somehow to involve synaptic connections, -- microscopic structural rearrangements in the synaptic membrane or submicroscopic transmitter-receptor site modification. The latter is exemplified by a recent experiment of Luco's with peripheral excitable structures in the nictitating membrane of the cat. After switching nerves and reinnervating with a cholinergic nerve, the nictitating membrane develops a high concentration of specific cholinesterase, whereas normally its concentration is low (Vera et al.,1966).

Franzisket (1951) paired a weak and a strong scratching stimulus to the skin in two different places in chronic spinal frogs. In every one of 16 specimens he finally obtained, to the weaker stimulus alone, the response typical of the other reflexogenic locus and stronger stimulus. He considered this to show conditioning. Controls seem to show that sensitization is not responsible.

Experiments of Chamberlain, Rothschild, and Gerard (1963) on lasting changes in the spinal cord of mammals are slightly closer to the familiar ideas of learning. Certain imposed stimuli (unilateral lesions of the cerebellum or vestibular system) cause a postural asymmetry in hindlimb muscles. If the spinal cord is then transected above the levels of nerve supply to these muscles, the postural asymmetry persists, even though the cord remaining below the transection is no longer receiving asymmetrical input, providing more than 45 minutes has elapsed after the initial lateralizing lesion. An earlier transection does not allow time for the descending asymmetrical input to become fixated. Further experiments of this group employ the fixation period in order to estimate the influence of drugs and compounds of interest in RNA metabolism.

Classical conditioning of the mammalian preparation caudal to a spinal cord transection was reported by Shurrager and Culler in 1940, but was received with considerable scepticism. Franzisket (1951,1963) found the spinal frog is capable of habit formation and conditioned reflexes. The unexpectedness of this finding depends on whether one is more impressed by the lesser plasticity of lower vertebrate classes compared with mammals or by the greater autonomy of their spinal cord. Both generalizations seem to be valid evolutionary gradients and yet they might appear to conflict in predicting learning ability of the cord.

Ongoing experiments by Buchwald and Schramm (1965) give fresh support to the idea that learning ability exists in the mammalian cord. One or two days after complete spinal transection at L_1-L_2 level, kittens received training sessions of 35 trials four times daily in which a light brush stroke on the skin of the leg (CS) was paired with an electric shock train of 0.5 sec to the paw of the same leg (US). At first, CS caused no response and US caused a brisk flexion; but after 4 to 5 sessions, all kittens (18 at the time of the Work Session) began to respond to the brush stroke and increased to 80%-90% of positive trials in subsequent sessions. Withholding US causes gradual extinction, while its reinstatement quickly restores the response. Control kittens given the sessions of CS alone also begin to respond after 4-5 sessions, but unlike the paired group with US withheld, show no extinction since they never received the US. Littermates with intact spinal cord show no consistent response to the brush stroke, either after sessions paired with US -- which produce a good flexion -- or with brush strokes alone. Transection of the cord per se can be said to lead to a new reflex, i.e., hindleg flexion to an initially neutral brush stroke. This reflex develops as a function of time after the operation more than number of trials. This progressive hypersensitization of the cord following transection is then the control or expected responsiveness in the spinalized kitten. The reversible change in reflex responsiveness is thus shown by the response's decline when a long-paired US is withheld, and its reappearance when the shock is reinstated. It illustrates a degree of plasticity not irrelevant in the classical conditioning paradigm.

Related studies of Buchwald et al., (1964) are interesting in another way. Analyzing the role of sensory input in learning, she found that deletion of all sensory inflow from the cat's hindleg by dorsal root section prevents the development of a conditioned flexion in that leg (animal not spinalized). But if a small amount of sensory innervation remains, conditioned responses are developed in the partially innervated limb. Study of the role of gamma motoneuron - muscle spindle feedback shows that it is especially important for conditioning. Paralysis by Flaxedil, a drug that blocks neuromuscular transmission, both from the gamma motoneurons to the muscle spindles and from the alpha motoneurons to the gross muscle, prevents the development of a conditioned motor response (tested in the unparalyzed state). But subsequent training of the same animal in the unparalyzed state shows more rapid conditioning than controls, i.e., some savings.

Presumably what is important in the motor performance is "conditioned" proprioceptive discharge from the muscle spindles produced by conditioned discharges of the gamma motoneurons.

Relevant to the same general problem are studies of Spencer, Thompson, and Neilson (1964) and Thompson and Spencer (1966) and papers referred to therein. These were not discussed at any length in the Work Session but are listed for convenience in the bibliography.

It seems clear that further exploitation of the spinal cord of vertebrates including mammals is worthwhile. This is a relatively simple system capable of relevant types of alteration with experience.

The Use of Lower Classes of Vertebrates

The use of lower classes of vertebrates, another major avenue of study, can be represented by ongoing studies of C. L. Prosser (who permits us to mention work which is only partly published (Prosser, 1965)) and collaborators at the University of Illinois. The preparation is a goldfish whose evoked potentials in the tectum of the midbrain are recorded in response to a flash of light, using semi-microelectrodes and summed deflections from many units. Pairing an electric shock (US) after many repetitions induces a second, later wave on the falling phase of the photic evoked deflection; with a few hundred pairings, the light flash alone (US) elicits the whole complex response. The second wave extinguishes gradually with light flash alone. The cerebral hemispheres are not necessary. Pairing is believed to be necessary, and backward conditioning is ineffective in eliciting the conditioned response. Note that CS after conditioning does not elicit the same response as US alone but the same response as CS + US after many repetitions.

At the Work Session, Agranoff's series of biochemically oriented studies on learned responses in fish represented the use of lower vertebrates. Though the whole unsimplified animal is used, the experimental design and the level of complexity of the effects observed made these studies appropriate to the subject of the conference.

Agranoff's general research design is to interfere with protein synthesis during various stages of learning, then test for retention of the learned task. In order to examine the effect of puromycin (a protein-synthesis inhibitor) on learned responses in goldfish, Agranoff established a simple condi-

tioning experiment using a shuttle-box with a shocking grid. (See Fig. 11.) The goldfish is placed in one side of the tank and given 20 seconds of light followed by 20 seconds of paired light (CS) and shock (US). The fish learns that when the light goes on it can avoid the shock by swimming over a hurdle in shallow water to the other side of the tank. After 20 seconds of rest there, the fish must swim back to the other side if it is to avoid another shock. When the fish swims over the hurdle in the presence of the light (CS) before the shock (US) goes on, it is scored as a positive response (avoidance). Each fish is given 20 trials daily, with 5-minute rest periods every 5 trials. It is then rested and run again 3 days later.

If the goldfish are injected with puromycin (90 mg) into the fat pad over the brain immediately after the 20th trial of the first day, retention of the learned response is reduced when tested on the third day. Intracerebral puromycin has been found by Flexner et al. (1963) to cause a similar deficit in mice. Puromycin amino-nucleoside, a substance similar in structure to puromycin but probably lacking the effect of inhibition of protein synthesis, produces no decrease in performance of the conditioned response when injected.

The mechanism by which puromycin interferes with protein synthesis is thought to be related to the structural resemblance of puromycin and the terminal end of transfer RNA. Experiments (Morris et al., 1962) have demonstrated that puromycin blocks protein synthesis by releasing incompletely formed peptides from the ribosomal surface. Agranoff confirmed that puromycin acts in this way. He tested for the effect of parts of the puromycin molecule by injecting the dimethyladenine and aminoriboside of the puromycin molecule as well as methyltyrosine; none gave any effect. He tried other inhibitors of protein synthesis, such as acetoxycycloheximide, and found a behavioral deficit. Phleomycin is a copper-containing polypeptide antibiotic which has been reported to inhibit DNA polymerase. Agranoff decided to use this for possible further information as to the role of protein synthesis in memory fixation. High doses are required but they might cause some loss of retention.

Further experiments on injection of the goldfish with <u>puromycin before training</u> revealed that the animals can learn, but after 3 days show memory deficit. The response is the same as when the same dose of puromycin is given right after training. This indicates to Agranoff that whatever component is involved in so-called short-term memory, it is not puromycin-sensitive. In answer to a question by Roeder, Agranoff stated

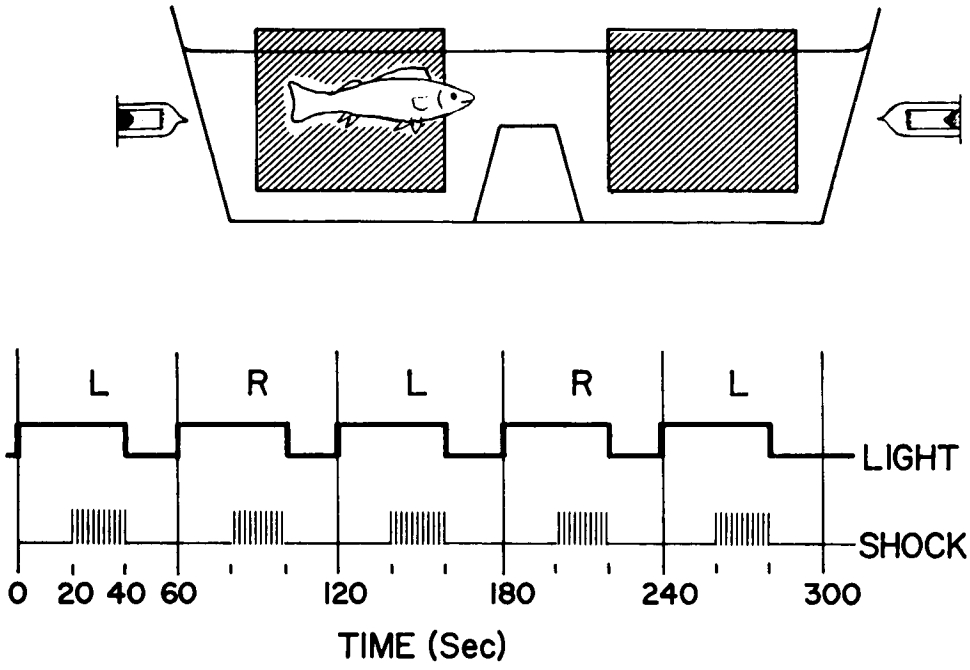

Figure 11. Diagrammatic representation of goldfish shuttle box and trial sequence. [Agranoff, Davis, and Brink, 1966].

that the puromycin is effective for 20 minutes before training and until 30 minutes after. He has not given puromycin later than 2 hours post-trial.

Agranoff next tried giving the fish electroconvulsive shock (ECS: 20v 60 cps AC, for 15 seconds as compared to 3v in the shuttle box) after the last trial. The effect is much like that of the 90 mg dose of puromycin -- interference with retention of the habit. If the ECS is given to a series of fish at different times after completion of the 20th trial, the interference with retention is less the longer the elapsed time; and if it is given more than 90 min (19°C) after the last trial, its effect is not significant. The fish are tested 3 days after the ECS, as in the original experiment, and those given ECS 2 hours after the 20th trial on the first day perform as well as the controls. There is no deficit after a painful but subconvulsive shock for 15 sec even if given right after the 20th trial on the first day. As control for a possible performance-decrement effect of puromycin, the drug was given both to fish that had been over-trained for many days and to naive fish 3 hours before their first trial. There was no evidence of performance decrement in either case.

A further experiment involved combining ECS with lowering the temperature of the fish. Goldfish become narcotized at 0°C or 37°C but a change of 10° either way from the normal room temperature of 19°C only causes them to swim more slowly. Four different treatments were tried with these results: (1) fish at 19°C with no ECS give conditioned responses in agreement with the predicted score; (2) fish at 19°C with ECS at 2 hours perform just as well as control fish in the first group; (3) fish cooled to 9°C immediately after the 20th trial for 2 hours and given no ECS retain as well as controls in the preceding groups; (4) fish cooled to 9°C immediately after the 20th trial and given ECS at 2 hours show a marked deficit in retention. The critical period is prolonged by cold (Ransmeier and Gerard, 1954); consolidation of memory is delayed.

The effects of puromycin and ECS are similar but the time during which memory is disruptible seems to differ with the two methods. ECS is effective for 2 hours in the chilled fish, the puromycin for only about 30 minutes.

McCleary asked whether he had kept the injected animals and tested them after a week or so of recovery. Agranoff has not done this with puromycin-injected animals but 2 weeks after ECS there is some improvement and a residual deficit.

Kandel suggested "simplifying" the goldfish, using the tail-flip response in which the Mauthner cell is involved. Thus the biochemical approach might be focused on two identifiable symmetrical cells whose electrophysiology is well known. The output of the Mauthner cell is involved in the tail-flip response which is an important motor act. Schmitt pointed out that the Mauthner cell has an electrical synapse. Bullock added that there is good evidence for both chemical and electrical excitatory and inhibitory synapses and a high degree of convergence and integration in the Mauthner cell.

Kandel, in response to Strumwasser's question about whether any behavior in the Mauthner cell is known to be relevant to learning, answered that the tail-flip response is known to habituate. Strumwasser emphasized that electrophysiologists should show Mauthner's cell is relevant to learning before bringing it to the chemists. Kandel thought that we should look for information relevant to learning at a level between the complex effector behavior in the whole goldfish on the one hand, and the pharmacological biochemistry of the learned act on the other; that is, we need the neurophysiology of the learned act. For this, the preparation needs to be simplified. Bullock agreed that it is important to localize the relevant events.

Agranoff felt that a phenomenon that he is disrupting by biochemical means occurs at some place in the brain; but this may be a biochemical rather than a geographical "place." He believes the time of the event, at least, has been found; perhaps a geographical locus can be found by ablation if performance decrements do not interfere. McCleary disagreed with this, saying that he has done shuttle-box studies with ablated fish; one can ablate everything back to the midbrain and the conditioned performance is just as good. It might be possible, Agranoff said, to place puromycin in various loci in the brain by micro-injection techniques, and also to use autoradiography for localizing an event. He hopes to do labeling experiments and subcellular fractionation to find out more about the role of protein in these phenomena.

VI. RELATED STUDIES ON TISSUE CULTURES

Crain reported on his studies of the electrical activity in tissue-culture explants of fetal rat spinal cord and neonatal mouse cerebral cortex.* After a few days in culture, both the cord and the cortex explants (about a cubic millimeter in size, and comprising about 1000 neurons) begin to display complex electrical activity with long-lasting responses to electrical stimulation. With longer periods in culture, the responses increase in amplitude, complexity, and regularity. The afterdischarges often include 10/sec rhythms which can be loosely likened to the alpha rhythm of the EEG.

Crain showed recordings from a fetal cord explant cultured for 3 weeks in vitro at which time oscillatory afterdischarges are often first seen. In this culture, however, complex evoked responses of relatively short duration (about 50 msec) occurred at two sites upon application of a single brief stimulus to a third region of the explant, all sites being several hundred microns apart. When two stimuli were given with a 100 msec interval between them, facilitation resulted in a longer-lasting, 2-cycle repetitive response. If one returned to a single stimulus, the response returned to the briefer, single-cycle pattern. About a minute later, dual stimuli produced a 3-cycle repetitive response, indicating a little more facilitation than before. Within a few seconds, a single stimulus now produced a much longer-lasting oscillatory afterdischarge than that evoked by the paired stimuli, the repetitive sequence often continuing for more than a second. This increase in excitability lasted for several minutes, and could be elicited again by re-application of dual stimuli. This phenomenon has been seen in several cord explants and Crain suggests that these plastic changes in excitability may indicate the potentiality of cultured CNS tissues for studies related to memory and learning (Crain, 1966).

Attempts to obtain comparable facilitation with heterosynaptic stimulation (applying shocks in two different places on the explant) have not produced such long-lasting alterations in excitability as those from the homosynaptic situation just described.

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* Both Crain's experimental techniques and his results (showing maintenance of functional integrity of the tissue cultures with bioelectric activity closely mimicking patterns found in the mammalian CNS) were described in detail in an earlier NRP Bulletin, Vol. 3, No. 4, "The Synapse" by J. D. Robertson.

Crain referred to work by Walter (1962), Brazier (1960, 1963), and others (Barlow, 1960; Hughes, 1964) in which single visual flashes produce in human cerebral cortex a primary evoked potential, a silent period of about 300 msec, and then a 10/sec rhythm lasting for several seconds. He noted that while it may be fortuitous, the network that develops in many of the CNS cultures can respond with a complex sequence of bioelectric activity after a single brief stimulus that is remarkably similar, at least superficially, to this human cortical pattern.

So far, Crain has not been able to obtain these complex bioelectric activities from explants thin enough to permit good visualization of the individual neurons and their processes; but he hopes to be able to combine these two qualities in one explant in order to study the electrical activity while watching the cells grow and organize.

An interesting additional note is that application to the culture of sera from animals with experimental allergic encephalomyelitis and from humans with multiple sclerosis causes rapid, reversible blocking of all the complex electrical activity discussed above, at levels where simple spike potentials can still be evoked (Bornstein and Crain, 1965).

Bullock challenged Crain to give his "best argument" for occurrence of something like learning in his cultures. Crain replied that, at this preliminary stage of the work, he can only talk about plastic changes in the excitability properties, lasting for minutes, which can be produced by simple combinations of input stimuli. One other aspect that might have bearing on learning, however, is a phenomenon which is frequently seen immediately after transferring an explant from the sealed Maximow culture-slide to the experimental electrophysiology moist-chamber. The first few stimuli often produce very mild or no responses; but then gradually, as additional shocks are administered at constant strength, the responses become larger in amplitude, longer-lasting, and more widespread. This might reflect learning, in the broad sense, of an altered responsiveness to the same input. He has not, however, been able to eliminate the physico-chemical variables in this experimental situation sufficiently to quantitate excitability changes which may be due to repeated electric stimulation of these previously quiescent tissues.

Bullock asked if complex responses from stimulation of the axons in the outgrowth region are interpreted as being an antidromic effect. Crain replied that these afterdischarges

may be the results of antidromic invasion of abundant collaterals from the stimulated axons, leading to activation of many other neurons through multi-synaptic pathways. It should be noted that many of the neurites in the outgrowth may actually be dendrites, since no clear-cut distinction from fine, unmyelinated axons has been possible. The fact that stimulation at any point in the culture gives a response whose delay varies only slightly from locus to locus suggested to Chorover that all parts of the culture are functionally interconnected. Crain said that in many of the older CNS explants, widespread, positive evoked potentials were commonly seen, which he interprets as extracellular indications of inhibitory postsynaptic potentials, although the only direct proof would be intracellular recordings.

Kandel noted that, by and large, these patterns correspond to what is seen when the mammalian brain is stimulated; recurrent inhibitory pathways are very extensive and powerful. Pharmacologic agents, e.g., strychnine, may convert the monophasic evoked potentials of these explants into long-lasting diphasic, oscillatory afterdischarges. Crain feels that his studies fit in with the current theory, as proposed by Andersen and Eccles (1962,1964), and others (Jasper and Stefanis, 1965; Purpura and Cohen, 1962; Stefanis and Jasper, 1964), of inhibitory phasing being a cause of the synchronized, rhythmic discharges, at relatively low frequency -- of the order of 10/sec -- of various aggregates of CNS neurons.

The repetitive discharges in CNS explants can be fractionated by lowering the temperature gradually to 29° C; the length of a sequence decreases but the pattern remains the same (Crain, 1966). Hoyle saw this response pattern as slowly rising to a peak. He mentioned Farley's 1962 work with computer models of randomly connected nets as a possible analog of the tissue cultures. Crain agreed, adding that when Farley (1965) constructs nets with a "loose" coupling and with relative refractory periods of the network units set at 10-20 msec, he can obtain synchronized, widespread bursts of 10/sec oscillations following a single stimulus to a few neighboring units. According to Crain, the refractory properties which Farley assigns to each element after it fires could also include active inhibitory processes. Hoyle pointed out that in Farley's work, the frequency increases with a finite time constant then decays to zero and stops until a new stimulus is given. Wilson stated that such a rhythm does not require an extensive net but can be obtained with only two interconnected cells with a reasonably long time constant of refractoriness or fatigue. Bullock added that not even the two cells mentioned

by Wilson are needed for periodic afterdischarge or for maintained rhythms. There are various cases of slower and faster afterrhythms in single elements as well as spontaneous rhythms of a wide range of frequencies. Crain pointed out that the complex oscillatory afterdischarges seen in the cultured CNS explants appear to involve integrated, synchronized activities of large numbers of neurons, with temporal patterns which closely mimic some seen in the intact brain.

Wilson reported that if electronic analogs of neurons are interconnected by means of excitatory synapses, oscillatory behavior tends to develop. The oscillations result if fatigue or self-inhibition in each unit builds up slowly at first but finally at a higher rate than the mutual excitation. The stronger and more numerous the connections made, the deeper the oscillations and the longer the period. He feels, therefore, that it is not surprising that something like the alpha rhythm is obtained from any piece of brain or nervous tissue; in fact, it would be surprising if it were not. This of course presupposes that the alpha rhythm is due to excitatory interaction between cells. To Kandel's question why an alpha rhythm occurs in the neocortex but a theta rhythm (4-7/sec) in the hippocampus, Wilson admitted that the properties of the connections and cells are important, too. The frequency of oscillation in the model networks depends on several variable parameters.

Crain noted that Bullock has pointed out for years the difference in brain waves between invertebrates and vertebrates; practically speaking, slow waves occur only in vertebrates (Bullock, 1945,1965). The patterns in Crain's older cultures simulate those recorded from the vertebrate brain, and he believes this indicates that these neurons are being connected in ways similar to those in the intact animals. During the first few days in culture, on the other hand, these immature CNS explants show only simple spike activity. Kandel remarked that he has been struck by the fact that in all mammals one finds strong inhibitory effects; he believes they underlie the slow rhythms in the mammalian brain. Wilson thinks this same pattern can be obtained without recurrent inhibition; there must be inhibition or some form of fatigue but it need not be recurrent (Farley, 1964). Bullock pointed out that the brain waves of lower vertebrates are extraordinarily like those of higher vertebrates, i.e., mammals. He wondered how we could assess the relative occurrence and importance of inhibition or of other features in such vastly different levels of complexity, some with and others without a cortex or neothalamus. He also wondered about the richness

of dendritic development and status and topography of synapses in Crain's cultures since these are often invoked as possible explanations of the difference between vertebrate and invertebrate ongoing electrical activity. This explanation may not be adequate in view of: a) the relatively modest development of dendrites in the cortex of very young mammals when brain waves with slow potentials are already found; b) possibly modest dendritic development in newborn mouse cerebral cortex explants after only 3-4 days in vitro where oscillatory slow waves can also be detected*; and c) the relatively good profusion of dendrites and telodendria in higher invertebrates.

* Synapses form in great abundance in the cerebral explants during this first week in vitro, on the basis of electron microscopic studies by Pappas (Crain and Bornstein, 1964; Pappas, 1966).

VII. CONCEPTS AND DEFINITIONS OF LEARNING PHENOMENA

Why Try to Define Learning?

An assumption underlying much of the work on invertebrate learning is that it is easier to do research on simple systems than on complex ones. This assumption leads naturally to a search for simple systems. However, it is important not to throw the baby out with the bath. In attempting to simplify, it is possible to designate as learning a phenomenon so different from learning in complex animals that nothing gained from its study can be applied to the more complex case. To avoid this, it seems important to try to define learning and to determine diagnostic learning characteristics that should be present in candidate systems if they are to serve as models for more complex systems. Schmitt, in particular, pinpointed the importance of this need, observing that many biochemists interested in studying the chemical changes accompanying learning have already been confronted with the difficulty of finding a definition to which psychologists will agree. They are concerned that they will identify chemical changes that psychologists will not consider to be relevant to learning.

Despite the apparent need, several of the Work Session participants agreed that it was impossible to arrive at a definition that would cover all the situations under study that are probably relevant to the biology of learning. A number of participants even felt that such a definition is not necessary at this stage of research on physiological mechanisms. Both Chorover and Kandel wondered whether it is possible to apply the fairly well-established concepts of conditioning and learning in man and other mammals to the behavior of lower animals in simplified systems. Kandel prefers to use the neutral term "analogs of learning" when he refers to phenomena resembling learning that take place in isolated invertebrate ganglia or simplified vertebrate preparations. Use of such terminology would encourage research on these analogs of learning and would suggest that much of the information gained would be relevant to complex systems, while not assuming an identity of these processes.

Agranoff's position, shared by some other participants, reflects some impatience with definitional matters. He advocated continued research on all aspects of the problems that seem intuitively to be important without at present placing too much emphasis on semantics. He argued, in effect, that we do not yet know enough to be precise in our definitions; he felt that too much concern with the labels we are using might

interfere with the more important goal of extending our observations.

A number of participants pointed out that our reasoning is somewhat circular in that we are attempting to define a phenomenon as yet inadequately described and then trying to use that definition both to extend the description and to provide an explanation for it. Edelman, although agreeing that circularity may be an obstacle to arriving at a precise permanent definition, felt it does not necessarily impede the development of a science. He pointed out that the present stage of learning research is somewhat analogous to that of immunology several years ago. At that time the classical immunological definition of "antigen" was that it is something which, when placed into an animal, will produce something called an "antibody." At the same time an antibody was defined as something produced when an antigen is injected. This definition, while circular, nonetheless enabled an enormous elaboration of experimental research to take place. Neither definition is now used because recent knowledge of the mechanism of the antigen-antibody interaction has made new and more precise definitions possible. According to Edelman, had immunologists waited initially for a perfect definition, their science would not have progressed as rapidly as it has.

Chorover also commented in the same vein, pointing out that we are really searching for a paradigm for use in studying behavior, not searching for a permanent definition.

Bullock felt that discussion of definitions would be useful despite the reservations of some participants. In his opinion, most investigators do, in fact, operate with unexpressed definitions; greater precision would result from making them explicit. Chorover felt that this group's problem in arriving at a definition was one inherited from psychologists, who themselves have had a great deal of difficulty in defining precisely what is meant by "learning." This difficulty is at least in part due to the fact that the phenomena thus subsumed have many facets and manifestations. Textbook definitions might be acceptable to people who are not primarily psychologists, but they are not adequate to designate the whole range of problems of interest. Chorover thinks it would be absurd to exclude by a narrow definition consideration of phenomena that have attracted real interest among workers in this field, such as studies on the habituation of the orienting reflex. He argued for an approach that would keep this and many similar topics within the range of interest of those studying learning in simple systems.

Kinds of Learning Phenomena

Of the several kinds of learning phenomena recognized by psychologists, (see Thorpe, 1963a) only a few were discussed at the Work Session, mainly habituation and conditioning (both classical and instrumental). Others, touched upon or referred to in passing, will be treated only briefly in this report as follows:

Imprinting phenomena elicited no controversy as aspects of learning; though there has been debate in the literature over whether they are a distinct class, there was no substantial discussion of their diagnostic features or mechanisms. Though the examples in lower phyla are not as well characterized as in birds, there are suggestive cases in insects. Imprinting is operationally simple and should be as suitable for study with respect to mechanism as the so-called higher forms of conditioning.

One-trial learning, described by Chorover in rats, also elicited no extended comment. It may be considered a special case of trial-and-error learning or instrumental conditioning, but this does not mean its mechanism is necessarily the same as the more-discussed cases requiring many trials.

Insight learning involves the production of a new adaptive response as the solution of a problem by the sudden reorganization of experience. It is a highly important phenomenon not only in man but in animals as low as insects. Examples in the latter group include nest repair by previously unused movements. Despite a general impression that it is less amenable to experiment than other forms of learning, insight learning remains one of many possible approaches to studying the mechanisms of learning. It could be quantified in cases like web-spinning by spiders with legs or claws amputated.

Latent learning (a term coined for cases not adequately explained by trial-and-error learning, and in which there is no patent reward, as in certain forms of maze learning), is a category frequently treated as distinct. This apparently unrewarded behavior is important in mammals and even in lower animals (crickets, butterflies, dragonflies, wasps, etc.), and is involved in the very natural task of becoming familiar with one's environs. While this form of behavior is certainly not conditioning, and might better be called "exploratory learning," Thorpe and some other writers have concluded that it can basically be related to insight learning.

Habituation vs. Fatigue

Habituation is quite generally accepted as a form of learning, although its manifestations in lower animals and simplified preparations are naturally quite rudimentary. Even in this simplest form of learning there is no reason to assume a single universal mechanism; for example, habituation of nystagmus in pilots or ballet dancers may not necessarily utilize the same mechanism as habituation in Aplysia neurons or in very primitive species. Its definition provoked considerable discussion. In Thorpe's formulation, (1963a) habituation is:

> "... the relatively persistent waning of a response as a result of repeated stimulation which is not followed by any kind of reinforcement."

This was understood to require a demonstration that the cause is neither in the receptor not in the effector, but instead is central. While both sensory adaptation and decline of neuromuscular transmission are thus excluded, there was disagreement about the role of central fatigue.

Following Tauc's presentation of his work with habituation in Aplysia (Section IV, above), there was some concern over the difference between habituation and fatigue at the neuronal level.

Chorover suggested the possibility of dissociating fatigue and habituation in the following way: Instead of using a different site and mode of applying the initial (repetitive) stimulus and the terminal (novel) stimulus, the two stimuli should be applied to the same receptors in the same way, but at different intensity levels. While a stronger terminal stimulus might produce the same effects in a fatigued and in a habituated system, a weaker terminal stimulus would be ineffective in a fatigued preparation but should produce a reappearance of the response in a habituated preparation. An obvious way to do this would be first to stimulate repetitively with water drops falling from a fixed height; the test (novel) stimulus could then be a drop falling from a greater or lesser height. Luco felt that it is not easy to establish the difference between fatigue and habituation. Bullock disagreed, saying that if Chorover's test showed dishabituation by a weaker stimulus, we could not speak of its being fatigue. The system as a whole is said to habituate because depression results from mild stimulation and is reversed by changes in timing, strength, or additional modalities that would aggravate

fatigue. Luco reiterated his point, saying fatigue in the CNS has not been studied. Thus we do not really know what fatigue is; habituation has been studied much more. In fact, said Luco, when he studied fatigue 15 years ago, it was defined as a situation in which either chemical mediator could not be released or the amount released by a nerve impulse was decreased; the same definition, he says, is now used for habituation.

Bullock pointed out that the definition cannot yet be based on a mechanism, but has to be operational; accepted distinctions between habituation and fatigue based on their properties can be found in the literature, for example in Humphrey (1930, 1933), Konorski (1948), Verplanck (1957), Thorpe (1963a), and in Thompson and Spencer (1966).

Hoyle argued that different mechanisms underly habituation and fatigue in the nervous system and that important physiological differences exist between the two. Kandel protested that we are in no position to specify what the mechanisms of these processes might be; habituation refers not to a certain process at the cellular level but rather to a response decrement with certain properties distinguishing it from primary input or output causes. He cited the response decrement in the intact preparation by Spencer, et al. (1964), who studied the withdrawal reflex to a noxious stimulus and showed that in this case disinhibition (the hypothesis proposed by Hoyle) is not a factor in dishabituation. Kandel remarked that this does not mean that disinhibition might not underly habituation in another system. One simply cannot define by mechanisms at present (Pantin, 1965). Bullock agreed and repeated that operationally, habituation is well-defined and distinguishable from fatigue and other kinds of response decrement. Eventually we may know all the mechanisms and will be able to reclassify; meanwhile, although it may be hard to apply operational criteria, they do exist. Sometimes, for example, habituation does not occur if a strong stimulus is used; the opposite is true of fatigue, which is more likely to occur with a strong stimulus. A habituated response may reappear upon a slight change in the form or timing of the repetitive stimuli, including changes that can exaggerate fatigue. Above all, habituation is quite specific to the stimulus. It is well-shown at long intervals and the response recovers with either shortening or lengthening the interval. Some stimuli are extremely resistant to habituation, although involving the same modality as others that readily habituate. It is said that one virtually cannot habituate the response of the auditory system of a beaver to the sound of cracking matchsticks. This resistance is not

typical of fatigue.

Definition of Conditioning

Conditioning presents less of a problem with respect to definitions. Classical, Pavlovian, or type 1 conditioning occurs when two different stimuli (the conditioned stimulus (CS), which in the naive subject does not elicit the conditioned response (CR), and the unconditioned stimulus (US), which does), are delivered in that order ("paired") repeatedly, under the experimenter's control, until the response occurs to the conditioned stimulus alone. Instrumental, operant, or type 2 conditioning, or trial-and-error learning occurs when some action initiated by the subject is followed by a stimulus with reward or punishment ("reinforcement") value; the subsequent probability of the action is thus altered.

Quarton suggested that the temporal features are so critical to classical and operant conditioning that perhaps they should be included in the definition. Chorover emphasized that knowledge of features allows one to specify additional aspects of the conditioning situations. Thus, there is more uncertainty in specifying the necessary conditions in instrumental than in classical conditioning, since in the former no stimulus can be identified as the eliciting event. One can set up a situation in which conditioning will occur, but one cannot know how it came about. In classical conditioning, one pays a price in speed of learning, perhaps, but more parameters may be defined; the experimenter, not the subject, controls the situation.

Kandel thinks the temporal sequence is important in instrumental as well as classical conditioning; whether the reinforcing stimulus immediately follows a response or is delayed affects the ensuing learning. Chorover agreed that there are delay-of-reinforcement gradients in both instrumental and classical conditioning; many of the principles are shared by the two. Indeed, in many kinds of instrumental behavior, such as avoidance, the response depends upon prior classical conditioning to noxious stimuli.

Chorover believes he could define all the necessary stimulus conditions for extablishing a classical conditioned response. While more terms might be needed to define classical than instrumental conditioning, the operational definition is not necessarily more complex. He agreed with Quarton that some statements about temporal factors must be included in the operational definition.

In terms of neurophysiological models, how does classical relate to instrumental conditioning? Kandel proposed that the two paradigms relate to each other as a simple neural transform in a monosynaptic pathway. In classical conditioning, the investigator, by stimulating nerves, evokes and controls two kinds of responses: those specific to pairing and those that are not. Similarly, in instrumental conditioning, where one is dealing with spontaneously occurring behavior, there are two kinds of response: a change in frequency contingent on the position on the stimulus in time, and a change that is not.

In the simplest case, assume that in a cell a synaptic potential exists of a certain size. As a result of pairing, this potential enlarges until it triggers first one spike and eventually two, three, or more spikes. The amplitude change of the postsynaptic potential (PSP) at that neuron's input manifests itself as a frequency change in this neuron's output and at the next neuron's input. If the cell tends to fire spontaneously, and the investigator reinforces such occurrences with a suitable temporal contingency, the frequency of spikes will also be changed. The possibility exists that in the very simplest case, certain kinds of instrumental analogs may, in fact, simply represent transforms of classical analogs.

Definition of Learning

Coming finally to the problem of defining learning in general, it may be helpful to the reader to insert in the report at this point some quotations from previous writers, although these were not discussed at the Work Session. The glossary of Verplanck (1957) defines "learn" as:

> "To exhibit a change in behavior between two successive exposures to the same environment that cannot be attributed to manipulation of drive operations, alterations in the environment, sensory adaptation, disease, surgical interference, physical trauma, or growth -- although the propriety of these exclusions may be questioned. /When we say that an animal learns, we are stating at the least that, other things being equal, some behavior now occurs in a situation in which it had not occurred previously, or that the behavior now occurring in a given situation is different from the behavior that occurred in the last occasion the animal was in that situation. The behavior need not change nor the situation, but the relation between them has changed. For an extremely stimulating and logical treatment of the possibilities see Haldane (1954)./"

Thorpe (1963a) says, "We can define learning as that process which manifests itself by adaptive changes in individual behavior as a result of experience..." He explains the operative words as well as the limitations of this and earlier definitions in a short discussion. Lorenz (1965) construes learning very broadly as any adaptive modification of behavior during the life of the individual. His answer to the definition problem is typical of some seriously interested authors who attempt to avoid it by essentially discarding the term. The only diagnostic feature mentioned is a subjective opinion (adaptiveness) and is not operationally determinable. The extreme inclusiveness, embracing modifications due to growth, maturation, aging, acclimation, sensory adaptation, muscular hypertrophy, disease resistance and other changes not "learned" in the ordinary meaning of the work, destroys its usefulness.

More fundamental is the issue posed by the common element of the definitions of Thorpe and Lorenz: the unqualified assertion that learning is adaptive. What these authors probably mean is that the ability to learn is adaptive and that most naturally occurring learned behavior contributes ultimately to the welfare of the species. But "adaptive" has no place in a useful definition, which must guide one in recognizing members of the class and in excluding nonmembers, since maladaptive tasks may easily be taught to men and other animals. Hilgard (1956) points out in this vein that while we are tempted to consider learning as improvement with practice, or profiting by experience, we cannot do this, since we know very well that some learning is not improvement and that the consequence of some is not desirable.

Learning, furthermore, is not merely change with repetition, since growth, fatigue, and some other phenomena also change with iteration. Hilgard therefore falls back on a definition by exclusion:

> "Learning is the process by which an activity originates or is changed through reacting to an encountered situation, provided that the characteristics of the change cannot be explained on the basis of native response tendencies, maturation, or temporatory states of the organism (e.g., fatigue, drugs, etc.)"

Hilgard's useful discussion of the exclusions should be consulted. We may add to Hilgard's list of exclusions temporary states such as those of acclimation, disease and the like, and changes attributable to the receptors or the effectors.

(We need not try to become precise on these or the other undefined terms in the definition.) He concludes that problems inherent in the definition are not a major source of disagreement among theories of learning. It is different, however, when concern is with opening the black box to investigate mechanisms, expecially simple systems. Just what instances are good or relevant and what cases must be excluded by definition is pragmatically important.

During the Work Session discussion, Chorover proposed the following definition, designed to embrace classical and instrumental conditioning and other forms of modifiability:

> "In general, learning is the ability to modify behavior through a combination or association of contiguous stimulus events, where the contiguity is determined by the temporal sequence of the events or their spatial relations and the origin of both events is external to the organism."

Argument on this definition centered around the final statement that both stimuli must be external to the animal. In classical conditioning they are patently external, but in instrumental it is less clear. Chorover explained that in the latter, both stimuli are external in that they demand an interaction between the animal and its environment. Strumwasser asked how Chorover could apply this definition to the situation in which, for example, an animal in constant light gets on a running wheel progressively later every 24 hours, manifesting its internal clock. When the animal is put on a light-dark regime, a complex response pattern that synchronizes with the light-dark cycle is seen; one of the components is internal to the animal: a free-running cycle in constant light. Chorover agreed that "internal stimuli" do intervene between the external stimulation that initially produced or entrained (phased) the running in the animal's normal habitat and the present performance of the task in constant light. However, while the timing of the response can be internal to the animal, the conditions that originally induced and entrained the running were undoubtedly the consequences of environmental time-givers. While conceding that the internal clock can be phased by external events, Strumwasser stressed that overwhelming evidence (1963,1967) shows that it can run internally and autonomously. Chorover then suggested that the special case of changing of light-dark phasing in endogenous rhythms does not conform to the restricted definition of conditioning that he was trying to use. He reiterated that in most situations where behavioral modification can be identified, there is an initial stimulus

and its consequence, which is another stimulus in an instrumental conditioning situation. The initial stimulus elicits some response, which itself provides sensory feedback indicating whether or not the given response has been "successful." Thus both events are normally consequences of external stimuli.

Chorover continued discussion of the definition of learning. One controversy that has raged in psychology concerns whether the learning function is a continuous process of accretion (as most learning curves would lead one to believe) or some sort of step-function that actually occurs in all-or-none fashion even though processes related to performance variables make it appear to be gradual. To Chorover, in many cases one can demonstrate learning to be essentially a step-function, occurring at a single trial as a consequence of a single experience. But in order to meet the various criteria for conditioned responses, people employ a large number of techniques to discern whether the animal's actions are unconditionally related to a given stimulus or whether the response has been learned. Various kinds of discrimination tasks are used that demand that an animal differentiate between two sets of stimuli (either one stimulus is reinforced and the other not, or one is accompanied by punishment and the other by reward). These are powerful ways of distinguishing between a generalized activation response to stimulation (essentially a pseudo-conditioning effect) and a specific discriminative response.

For learning to take place there must be stimulus association, either temporally or spatially defined. Only certain kinds of stimulus-response patterning will produce conditioning; reversing the order of conditioned and unconditioned stimuli (backward conditioning) works only in rare instances.

Eisenstein presented his definition of conditioning, saying that it is important to keep the definition as broad and as far removed from the arbitrariness of the procedures used as possible. He defined conditioning as:

> "...the ability to code and retain different patterned sensitivities to the same stimulus."

It may not be meaningful, on a molecular level, to speak of an experimental and control group of subjects in a test of learning, as if one demonstrated conditioning and the other did not. Both groups, the forward-conditioning and the backward-conditioning one (commonly used to control for such things

as sensitization and facilitation) may be actually coding differently with the same molecule (for example, RNA). He suggested that a system capable of coding differential outputs (i.e., responses) to the same test input (CS) as a function of the previous total stimulus-response pattern the system was exposed to during training, is one demonstrating conditioning. In both types of conditioning, (i.e., instrumental and classical) the temporal pattern is important, and best distinguishes the experimental from the control groups.

According to Eisenstein, any system, from a single cell to man, can be said to demonstrate conditioning if it meets the above criteria. To demonstrate the phenomenon the minimum that is needed is an input and output, where the output is a certain function of the input. This definition of conditioning (Eisenstein would even say, of learning) avoids a lot of questions he considers to be side issues. For example, can learning occur in a molluscan or arthropod ganglia. He thinks it simplifies the problem of control, since one does not have to say that one experimental procedure leads to conditioning while a control procedure does not. One says, instead, that a system learns if the two different procedures, experimental and control, produce a different retained code to the same input. Eisenstein stated, in summary, that the storage of patterned sensitivity is learning, while storage of changes to a given input indifferent to its previous pattern of presentation is not learning. He feels that ultimately it is a question to be answered by experiments alone whether molecular changes produced in a system sensitive to input-output pattern are the same or different from those produced in a system which is not pattern sensitive. (Learning may differ from facilitation in that the former represents a pattern-sensitive phenomenon whereas the latter does not). The fundamental question, he feels, in asking about the molecular mechanisms underlying learning,is how an essentially temporal sequence of stimulus and response elements are coded in some 3-dimensional structural charge within the system being investigated. (For further elaboration of this point see Eisenstein (1967). It is an empirical question as to what relation any such mechanisms uncovered in isolated systems might bear to the intact organism and to other subsystems (i.e., parts of the CNS) within the same organisms or to those higher or lower on the phylogenetic scale. The advantage of such a formulation, though, must be that it allows the investigation of progressively simpler systems where the chances of establishing mechanisms are greater.

Bullock remarked that aside from problems of accomodation, imprinting, one-trial learning, insight learning, and so on, one feature of Eisenstein's definition is that it is compatible with viewing learning as a spectrum of phenomena possibly unexplainable by any one mechanism; this could be very important to people who would like to find an answer to learning. He felt that whatever the popular conception, we are still dealing with a diverse set of phenomena, for which probably there will not be one answer or code. To him Eisenstein's definition is powerful even though it may not be coincident with the popular idea of learning. Agranoff was dissatisfied with this notion. He agrees with Schmitt's suggestion that learning be compared to genetics, as something definite that happens in nature. He does not think we are justified in changing what is a popular conception of learning as a natural phenonemon. Kandel suggested that this argument could be turned around, using Schmitt's argument in another way, to say that very frequently progress is made by changing the popular accepted definition.

Agranoff preferred to talk about models of learning in connection with work described as studies of learning. He noted that in studying metabolic pathways, model substrates are often used to find something known not to exist in nature. While artificial substrate reaction tells one something about the actual enzyme and the active site, it is never confused with the physiological process itself. The various chemical and other studies of behavioral changes are all valuable tools but should not be confused with the phenomena about which we really want to learn.

Kandel agreed with Eisenstein's definition, which he thinks is actually implied in much of the work discussed. Further, if those studying many different preparations could agree to the stimulus sequence and the response criteria, it would then be possible, for example, to see whether similar biochemical mechanisms are associated with all of them.

Chorover felt that a definition like Eisenstein's would suggest a reinterpretation of a large mass of behavioral data. This could give it tremendous strength, but only if it simultaneously took into account electrophysiological as well as behavioral data. He cautioned against the danger of letting the conceptualization of any given problem be a function of what we would like to see related to it. Obviously it would be advantageous to people working on these problems if the adopted definition of learning encompassed the phenomena completely. However, this might create the impression that

phenomena so encompassed under the definition, have, in a sense, been accounted for. Bullock felt a problem like that of neologisms versus excessively broad categories could be avoided by using adjectives to designate different learning phenomena.

Luco considers learning to be an instance of a more general phenomenon, plasticity. He thinks that the nervous sytem is a possessor of a "being" and of a power "to become." As the being increases, the becoming diminishes. The becoming is a potentiality that can be actualized by an appropriate stimulus. The potentiality of becoming depends on genetic factors. The stimuli for actualization are either genetic or environmental. Only when an environmental stimulus actualizes a potentiality, does the process of plasticity take place. Once an actualization has been achieved, two possibilities can be considered: the latent act and the present act. A man cannot see and cannot learn how to see light at certain wave lengths for genetic reasons; he has no potentiality for doing so. But a man can learn how to speak in a new language. Once he has learned it, he can either be using the language (present act) or he can be not using the language (latent act). Luco thinks that between potentiality and actualization a process is indispensable; but between latent act and present act only a trigger stimulus is necessary.

Eisenstein rephrased Luco's statement to say that there are input-output systems whose output is a genetically determined or relatively fixed function of the input (e.g., a reflex) as opposed to learning situations, for example, where the output is not genetically determined and can be varied, or even made to occur to an input which initially did not produce it. But Strumwasswer advised using the term "genetically determined" more carefully. There is essentially no mechanism by which the usual environment can alter or escape the genetically limited range of possible phenotypes. It can only switch on or off, up or down, what exists within the broader or narrower range. Strumwasser considers it exciting that genetic models in cells are being considered, but pointed out that they have grave limitations. In a genetic system dynamically operating in a cell, the only information of a novel type that can be stored is the time a change occurs; no new information can be stored.

VIII. EXPERIMENTAL DESIGN IN ESTABLISHING LEARNING PHENOMENA

From the psychologists' point of view, those who wish to demonstrate the equivalence of electrophysiological and behavioral phenomena or the chemical correlates of learning need to be fully aware of the various controls and parameters that play a role in establishing such equivalence.

Therefore, in this section a digest and extension of remarks by Chorover, Eisenstein, McCleary, and Quarton in particular is given, to aid the practical laboratory worker in determining whether an apparent instance of conditioning in fact meets the psychological criteria. The attempt has been made to use operations that depend on a clear definition. The goal of the decision is a categorization without arguing that a particular category is more important than any other.

Classical conditioning (see preceding section for discussion) has been defined in a standard psychology text as:

> "...An experiment after the prototype of Pavlov, which consists in the repeated presentation of the conditioned and unconditioned stimuli in a controlled relationship so that there occur alterations in reaction tendencies with respect to the conditioned stimulus which would not arise except for its relationship to the unconditioned stimulus and response. Distinguished from instrumental reward and escape training and from avoidance training in that the conditioned response neither delivers nor prevents the appearance of the unconditioned stimulus." (Hilgard and Marquis, 1940.)

However, this definition is not sufficiently precise to use in deciding whether an alleged instance of classical conditioning in fact is one. In particular, the nature of the relationship between the presentation of the conditioned and unconditioned stimulus is ambiguous.

A more complete working definition might be given in this way:

> A stimulus class (CS) that before the conditioning procedure does not produce a response, or produces it with a low incidence will, after the conditioning procedure, produce that response with a frequency above some specified criterion. The conditioning procedure consists in pairing of the CS with another stimulus (US) that ordinarily produces an

unconditioned response (UR). The paired presentations must be in a certain order (CS - US) with a CS - US interval within specified limits both with respect to mean duration and variability. The CR must meet criteria of resemblance to the UR.

Sometimes additional criteria are also employed, specifying limits to the development of the response as trials are administered, especially regarding number of trials, duration of learning period, and endurance of the response in the absence of reinforcement.

Violations of single conditions specified above, or of combinations of them, lead to events of organism-environment interaction of two major classes:

a) the apparent CR increases in frequency (false positives);

b) the apparent CR does not increase in frequency.

Only the false positives are likely to be confused with true classical conditioning. However, they can be classified in an orderly way by specifying exactly which condition or combination of conditions was violated. Three of these false positives, which may be biologically and/or psychologically important, are described below:

a) "Sensitization" is a term given by Wendt (see Hilgard and Marquis, 1940) to describe a response that increases in frequency when the CS is paired with the US, but does not resemble the UR. (Hull has called it "alpha conditioning.") It is one form of "nonspecific" enhancement of the alleged CR by a procedure resembling classical conditioning.

b) "Backward conditioning" occurs when the usual order of CS - US is reversed so that the US precedes the CS, but the US - CS interval and variation meet the usual criteria for conditioning.

c) In "pseudo-conditioning" the usual order is sometimes altered and the CS - US interval is essentially random in duration within limits. (Crether, see Hilgard and Marquis, 1940, page 348). Often the US is given alone several times and the response to the CS is greater than it was originally.

If backward conditioning occurs, or if temporally random occurrence of the reinforcement produces the same effect as classical conditioning procedures, the enchancement of the response has been obviously "nonspecific" in a sense different from that for sensitization. It is very important to note, therefore, that a number of different types of "nonspecific" enhancements of responses may occur yet not fulfill the requirements for conditioning.

All of the conditions specified in the working definitions given above are not of equal importance. Many authors do not include all these conditions in their definitions, and we intuitively allow minor violations to occur without excluding potential instances of classical conditioning. Optional requirements of this type pertain to the exact 1:1 pairing of CS and US, and to the time-course or trial-number conditions. While fulfillment of these conditions is not mandatory, a clear specification of such conditions in actual experiments is desirable, if only to make the procedure replicable, and to allow the reader to evaluate them for himself.

Let us now run through the steps that demonstrate that an apparent instance of learning meets the conditions specified for classical conditioning:

a) Identification of events that could serve as the components of the model; assigning values in criterion levels, etc. In practice, the events to be called "CS," "US," "CR," and "UR" must be designated. Further, the CS - US interval must be fixed, and the following other conditions must be specified:

   1) the direction and variability of the CS - US interval;

   2) the degree of resemblance required between CR and UR;

   3) the required change in frequency or pattern of the UR;

   4) the pattern over time or over trials, and the extinction-course characteristics, if these are to be included.

   Obviously some of these choices must be arbitrarily made for a given case.

b) Demonstration that the CR meets the CR - UR resemblance criterion. The CR must satisfactorily resemble the UR if sensitization is to be ruled out.

c) Demonstration that in the absence of any one of the specified factors, learning does not occur. Controls are used experimentally to demonstrate that full conditioning cannot occur in the absence of conditions postulated to be necessary.

This can be shown in two complementary ways:

1) Using the same stimulus (the alleged CS) in two ways. The CS is presented to one subject group in the classical way while to another it is presented with one or more of the conditions altered (such as reversed order of CS - US presentation, or random occurrence instead of pairing).

2) Using an additional and different stimulus from the alleged CS, and presenting the alleged CS according to the classical procedure. The additional stimulus is presented as often as the CS, and even during the same training period, but with some condition of classical conditioning violated, such as pairing with the US.

These two experimental procedures have the same goal, but they raise different practical problems. The first requires that the populations be equivalent on which the alleged CS is used in different ways. This is usually done by matching of the characteristics of the two populations that might be different and relevant. The second procedure raises an additional problem: stimuli may differ in their potential for conditioning. To validate the control procedure chosen, it is desirable to show that the two stimuli (the alleged CS and the "different stimulus" applied during the same training procedure) are both equally conditionable. The usual way of doing this is to treat one stimulus as CS for one population and the other stimulus as CS for another population. Only if the stimulus treated as CS in each population gives a CR according to criteria and the stimulus treated as a control in both cases fails to do so, can the control be considered adequate. Kandel used method (2) in

his experiment showing what appeared to be an analog of classical conditioning in Aplysia, (see p. 141) but he did not demonstrate equivalent conditionability of the control stimulus and the CS. Several participants pointed out that this would have been desirable or that, alternatively, he could have used method (1). It is not necessary to emphasize that an experimenter is not always free to use a desired method; difficulties inherent in the preparation, in equipment, or limitations of time may make such demonstrations difficult even when they have been carefully considered.

Most participants agreed that ruling out pseudo-conditioning is particularly important in studies of simple systems. However, the relatively non-specific enhancement that would probably account for the phenomena in pseudo-conditioning might be important biologically and psychologically and might be part of the mechanism used in assembling the more "advanced" mechanisms of true conditioning.

d) Demonstration that no factors other than the ones specified in the model could account for the change. This demonstration is much more difficult than the positive demonstration described in (c), because the problem is to rule out the operation of initially hidden factors. There appears to be no way of proceeding logically in this demonstration. The problem is to think of factors that might simulate the conditioning phenomenology and then look for them one by one. One can never be sure of having exhausted all possibilities.

As learning becomes more complex, closer to the familiar and well-studied, closer to the mammalian forms with which we have best rapport, it is intuitively less plausible that some hidden change could account for the phenomenon and mimic learning. With simple types of learning, or examples in systems that have been harshly treated (surgery, food deprivation, etc.), or in species with which we have less rapport, it is quite likely that some change produced in the organism or environment that is not learning might account for the observed change in response. These factors can, for practical purposes, be divided into environmental and organismic factors.

1) Environmental factors, not much discussed at this meeting, have been reported as possible explanations of alleged learning in protozoa, (Grabowski, 1939; Jensen, 1957, 1965). The argument runs that the treatment of the organism causes either a change in the environment (such as local CO_2 or temperature) or produces the proximity of other individuals that can modify response rate and mimic learning.

2) Organismic factors are even more diverse and more difficult to categorize, anticipate, prevent -- even to deal with theoretically. Common sense and English usage more than logical necessity compel us to exclude from learning those behavioral changes that can be attributed to aging, maturation, acclimation, change in satiation, state of deprivation, motivation, stress, attention, arousal, circadian and other rhythms, fatigue, changed activity level, modification of receptors or effectors, miscellaneous unknown effects of the surgery used to produce a simplified system, etc. Some of these have been mentioned above -- and were stressed in the Work Session, especially by McCleary.

Control procedures mentioned in the literature or considered at this meeting, once such a factor is considered plausible, consist in the experimental manipulation of the factor during a conditioning experiment, if it can be brought under experimental control. If the factor does, in fact, modify behavior in a direction that suggests it could simulate learning, it may be differentiated by comparing the response over time, over trials, or the post-treatment time or trial pattern with those of conditioning. For instance, maturation may influence a response rate that could also have been produced by conditioning. Although maturation cannot be prevented, the temporal pattern of its effect on the response can be studied both in the absence of the conditioning program and in its presence at different stages in the maturational process.

Similar methods of demonstrating the independence of conditioning from other influences on behavior may be possible when the alternate

influence is identified, but there is an additional problem in identifying such factors. A number of participants, particularly McCleary, emphasized the importance of knowing the whole behavioral repertoire of the experimental animal's species if interpretation of learning experiments is to be useful. It is obvious that the behavior of relatively little-known invertebrates may be influenced by unsuspected factors, (e.g., intrinsic tidal rhythms, gonadal state, nutritional state, pheromones) and that the surgical manipulations involved in simplifying systems create preparations whose behavioral repertoire may be influenced by factors we have not yet identified.

e) Demonstration that the apparent effective stimulus is, in fact, the effective stimulus. In experiments involving learning by complex organisms in complex environments, it is not always certain that the input the experimenter considers to be the effective input is, in fact, that used by the learning organism in the modification of its behavior. Several possibilities must be considered:

1) The organism reacts not to the input identified by the experimenter but to some concomitant event (e.g., noise of switches or camera shutters) possibly mediated by an unsuspected sensory channel.

2) The stimulus is apparently simple, but in fact the animal is influenced by some subset of components in the stimulus display.

3) The effective components of the stimulus situation drift during the experiment so that late in the learning trials behavior is influenced by inputs different from the initial ones.

4) There is an "occult tie" between stimuli so that the effects of one are effects of another even without pairing.

These considerations are important in learning experiments because if we do not identify the effective stimulus, it may be present when we believe it to be absent and vice versa. The conditions specified by a learning model therefore may not be

fulfilled. When we consider classical-conditioning experiments from this point of view, we must examine the alleged conditioned stimulus, the unconditioned stimulus, and any control stimuli to ascertain the effective component.

Actual experimental procedures that demonstrate the effectiveness of the identified stimulus cannot help but use a negative argument:

1) Stimuli that could originate from instruments or from personnel preparing or conducting the experiment may be looked for and ruled out by prevention, masking, or investigation of their effects.

2) Stimuli that can be simplified by using only part of a display can be studied by comparing the effects of the whole and partial displays.

3) Drift in stimuli can be looked for and analyzed experimentally, if possible.

4) Occult ties can be tested for by applying single stimulus categories and looking for effects on other stimulus-response patterns that have not been elicited. This problem may sometimes be equivalent to a search for "nonspecific" stimulus effects.

f) Demonstration that the apparent response is the one actually influenced by the learning procedure. Since the behavioral repertoire of an organism is greater than is self-evident from study of an arbitrarily identified action, unidentified responses may actually be conditioned. For example, Luco showed (Section III, above) learning to clean antennae in the amputated cockroach is actually learning to stand on three legs. This problem is perhaps not as serious for classical conditioning as it is for instrumental conditioning, since in the former the timing of the reinforcement is not dependent on correct recognition of the response, and any response that appears to be conditioned by the chosen criteria is probably conditioned in an important sense, even if it is not the only response that has been conditioned. It is possible that this problem is more relevant in the case of

> false negatives in which we mistakenly believe no conditioning has occurred, because we have not identified the response that has in fact been conditioned.

Much of the Work Session discussion of controls for sensitization and backward conditioning came during the presentation of Kandel, who believes that one cannot discard as irrelevant any alteration in behavior seen in the backward-conditioning test (US precedes CS); he views backward conditioning as a perfectly respectable specificity to pairing, and therefore a kind of conditioning. Eisenstein replied that there is an important distinction to be made between effects that are due to storage ability based on temporal patterning, and those summation effects due to increasing numbers of stimuli impinging on the system. He asks of the backward-conditioning test not whether one procedure gives conditioning and the other does not, but whether the system is able to make a differential response to the same stimuli as a function of their sequence. Although using a clear operational distinction between them, he asserted that since the underlying mechanisms are unknown, we are in no position to claim that basically different mechanisms underly backward conditioning, forward conditioning, and sensitization. For example, if RNA is the molecule coding behavior, it may be simply coding differently for backward and forward conditioning and sensitization, while using essentially the same mechanism. Kandel agreed, saying that he placed less emphasis on the phenomena that show specificity than on those that do not, since the latter are more easily studied. He feels that while specificity is very interesting and psychologically very important, a long-lasting phenomenon that can be specified at the cellular level, even though nonspecific, may nonetheless prove to be quite relevant. It is likely that the specific phenomenon may partake of some of the mechanisms of the nonspecific one, either by complex neural circuitry or by cellular change.

IX. DISCUSSION OF HYPOTHESES AND INTERPRETATIONS

Hoyle introduced a discussion of two alternative hypotheses for the organization of patterned performance that might be called the "motor-tape" and the "sensory-tape" hypotheses. According to the motor-tape idea, the animal preprograms its motor commands. Thus, detailed peripheral feedback confirming that each movement has in fact been performed is unnecessary. It assumes, speaking anthropomorphically and in terms of evolution, that certain patterns of efferent impulses will achieve certain movements. By contrast, the sensory-tape idea assumes that the animal uses an error-operated system in which impulses in proprioceptive afferent nerves inform the central nervous system of peripheral events; this input is then compared with a stored information pattern (sensory tape) of what is desired, and any mismatch results in corrective output.

The same issue arises in species-characteristic, unlearned, or instinctive behavior. Over the last several years Hoyle has been studying patterns of neural activity during locomotion, mating, egg-laying, and other acts in insects; his data indicate that the cricket's singing is motor-tape operated, while egg-laying, courtship movements and certain other actions are much more dependent on proprioceptive feedback (i.e., sensory-tape operated). Wilson's (1961) data show that flight movements in locusts are motor-tape operated. For most activities in most animal species, however, we do not know which system is used. When a spider spins its web, does it switch on a tape that automatically sends out commands in a fixed pattern, or does it start in some way and continuously compare the actual input from its leg receptors with a stored tape of the sensory input it should be receiving from the growing web? Data is generally not forthcoming, especially for learned movements.

Instinct vs. Learning

Hoyle supported the hypothesis that the mechanisms of innate and learned behavior are basically the same. According to him, work upon one of these, for example motor vs. sensory tapes in instinctive actions, is immediately relevant to the other -- for example homing or shock avoidance.

The proposition that these two broad categories of behavior are basically similar, while certainly never neglected, deserves new attention. It is, for example, diametrically opposed to the position of Lorenz (1965) whose book should be

consulted for the contrary arguments and references. He provides a stimulating and vigorous defense of the distinction between evolutionarily selected, genetically fixed behavior and that selected and fixed during the life of the individual as a result of its experiences. Lorenz provides a critique both of alternative views of "behaviorists" and "ethologists" and of the value and limitations of the deprivation experiment to test the hypothesis that a given activity is innate. His arguments are cogent and relevant to the present conference inasmuch as we have struggled to define learning, and have in large measure ended up defining by exclusion. Hebb (1949) and others have questioned the validity of the dichotomy of behavior into innate and learned on the ground that each can only be defined by the exclusion of the other. Attacking this position, Lorenz argues that both are defined by the sources of the information that is fed into the organic system. By interaction of the species with its environment during evolution, and by mutation and selection (trial and success), the species gathers information and stores it coded in the form of chain molecules and its genome. By interaction with the environment, the individual acquires information and reacts in two ways: one is the immediate response to stimuli according to inbuilt programs without changing those programs; the other is the usually adaptive modification of the machinery. We may quarrel with this unduly simple division, (which results in an unreasonably broad definition of learning as any adaptive modification of behavior) since this lumps together with bona fide learning the more doubtful "learning" phenomena of acclimation, maturation, healing, setting of biological clocks, and the like. Apart from this quibble, which he feels could sharpen and augment Lorenz's argument, and his objection (discussed elsewhere, p. 171) to the over-dependence upon the word "adaptive," Bullock, among others, feels the thesis of Lorenz's book is eminently justified and places the distinction between the innate and learned on a solid basis.

The relevance to the meeting goes further than this. As Lorenz has stated:

> "Many modern ethologists, particularly publishing in English, contend that the term innate is not only useless but heuristically harmful. They assume that phylogenetic adaptation and adaptive modification can be added to and mixed with each other in any behavior mechanism however minute and elementary. For this reason they regard it as hopeless and even dangerous to try separating, in experiment or thought, innate and learned elements

> of behavior. Even if, for example, a stickleback lacking all previous experience with a rival fights a model which is red underneath at first sight, this behavior cannot be called innate because some of its components and prerequisites, such as swimming movements or point discrimination on the retina, may have undergone adaptive modification during ontogeny."

Sharply challenging this defeatist and uncalled-for position Lorenz continues that the deprivation experiments need not be concerned with all the prerequisites of normal phenogeny so long as they do not contain the information whose source is being investigated:

> "Whatever wonders epigenetical phenogeny may perform, for instance, in the ontogeny of a stickleback, it cannot possibly extract from the factors indispensable for healthy growth (light, oxygen, sufficient food, etc.), the information that the rival that must be fought is red on the underside."

He goes on to criticize another view which amounts to an illogical mutual exclusion of the innate and learned. Finally, Lorenz offers rules for ensuring that a deprivation experiment will clearly provide an answer. If the information clearly contained in the behavioral adaption to an environmental given is made inaccessible to the individual's experience, and if under these circumstances, the adaptedness in question remains unimpaired, we can assert that the information contained in the genome (i.e., the behavioral pattern) is innate. The rules are interestingly reminiscent of those given above for establishing a behavioral pattern as belonging to a certain class of learning.

All this makes the issue particularly intriguing and provocative whether or not, as Hoyle asserted, the basic physiological mechanisms of innate and learned behavior patterns are actually similar. The similarity, of course, would apply, if true, to the storage and readout mechanism but not to the mechanism of acquiring and placing in storage. Certainly, as Bullock and others at the Work Session agreed, we need detailed knowledge of the way in which distinctive patterns are represented in the persistent mechanisms of the nervous system. In the case of learned patterns, this would be of some, and perhaps very large, relevance to the design and testing of candidate mechanisms for memory traces.

Structure vs. Activity; Localization vs. Diffuse Distribution

According to one traditional dichotomy, the changes in nervous tissue responsible for learning may involve either a structural alteration in a certain place, or a dynamic shift of ongoing activity in defined groups of neurons (Pringle, 1951).

The first alternative is often said to have been ruled out by Lashley's (1929) experiment on ablation of different regions of the cerebral cortex in rats. He reported that memory was impaired by a large lesion in a given area, although it survived a smaller lesion in any part of the same cortical area. The second alternative, dependent on the notion of continuous circulation of activity in neuronal circuits, is often thought to be excluded by the survival of habits through convulsions, hibernation, and even freezing.

The relevance of Lashley's experiment to the first hypothesis seems rather direct, although there have been criticisms that essentially limit its applicability to the particular species (rat) and task (maze-learning) used by Lashley. Other criticisms, which abound, pertain primarily to his interpretation in favor of "equipotentiality," but do not apply to the present argument. Criticism that individuals use multiple clues in different modalities and parts of the brain does not undermine the conclusion above that this task cannot depend on a grossly localized "engram" (persisting neural counterpart), though it does imply that separate constituent memories may be so parceled. However, the most cogent criticism in the present connection is that while in search of the engram we must also admit that these experiments could be explained by a well-localized memory trace outside the cortex and in nonspecific effects of the cortical lesions ("mass action"), such as motivation. The first class of hypotheses cannot really be said to have been ruled out, although there is a large body of evidence that seems to imply that specific memory traces apparently are not stored in very localized sites.

The relevance of the convulsion and cold experiments to the second class of hypotheses is also questionable. Admittedly, we do not know by direct evidence that all relevant activity is destroyed or silenced under these conditions. However, a number of participants in the Work Session did not lean upon this argument. Some, like Chorover, felt that the circulating activity hypothesis should be considered as having been excluded. Others, like Wilson and Hoyle, felt that it could be formulated to permit the possibility of temporary

silencing. It is not essential to Hebb's hypothesis (1949) concerning cell assemblies or to Pringle's (1951) involving coupled oscillators consisting of loops of neurons, that the activity be uninterrupted. These models assume that changes in both the internally generated ongoing activity in each of many neurons, and in the synaptic transfer functions, enable neurons to influence other neurons by degree. Both of these can rest upon properties that could persist during silence or convulsive periods. Chorover argued that these are then basically structural. The others replied that while this is true on an ultramicroscopic level with respect to molecular features that determine excitability and spontaneity, these changes need not be localized at an identifiable site in a gross or microscopic sense but may be distributed. The circuit of neurons could thus be the least element representing the stored information.

The foregoing refers to the degree of localization. There was less discussion concerning the degree of redundancy; it was apparently assumed that memory engrams are diffuse by reason of wide distribution in reduplicated traces. Lashley (1929) concluded that: ". . the equivalence of different regions in the cortex for retention of memory points to multiple representation. Somehow, equivalent traces are established throughout the functional area . . . perhaps in complex patterns of reverberatory circuits, reduplicated throughout the area."

There seems to be little positive evidence in favor of such specific reduplication in the sense of true redundancy, and Lashley's equivalence of different areas of cortex in the rat maze-learning experiments can be interpreted in at least two other ways, as mentioned above.

In discussing the actual changes with learning, Kandel re-emphasized that even if a completely biochemical mechanism of storage exists (which he considers likely) it will ultimately manifest itself again in electrophysiologically demonstrable terms. Schmitt agreed, but took issue with Kandel's statement that the closer one works to the electrophysiology of the nervous system, the closer one is to its total analysis. He doubts that electrophysiology can ever succeed in fully interpreting basic mechanisms, for the bioelectric parameter, including signaling, is itself the product of more elementary, yet unknown biophysical and biochemical processes at molecular and submolecular levels. Edelman likened the electrophysiologist's nervous system to a field operational amplifier; one

must understand the components if one is to discover the principles on which the total assemblage works.

Neural Correlates of Memory Trace

Most workers would probably agree at the present with the minimal statement that the neural correlate of the memory trace must involve structural changes, (defined in the broadest sense) including either microscopic (microns), submicroscopic (dimensions of membranes, patches, holes, clefts, etc.) or molecular (species, configurations, etc.) levels. These changes represent the memory only when considered in a total pattern distributed through some volume of nervous tissue and number of neuronal units, with some redundancy of greater or lesser degree in different cases. Each of the three levels referred to seemed highly probable to some of the investigators.

Schmitt raised the questions of the minimum essential circuitry, the locus of learning in the net, and the extent of the interconnection in which some kind of learning occurs. Hoyle answered that one could start by ruling out the cell bodies, at least in insects, since all the transactional events occur in the dendritic elements. Although this provoked a general discussion, Schmitt received essentially no answer beyond this.

The substance of the participants' discussion of molecular possibilities forms a separate section below (p. 195).

Contemporary evidence at the light-microscopic and electron microscopic levels certainly does not rule out changes in diameter and number of fine branches of dendrites or axons; in number or area of synaptic knobs, contacts, glial relations, membrane appositions, etc., or in their orientation with respect to one another. In fact, this offers one of the most hopeful areas of fresh investigation, ripe for quantitative treatment by electron microscopy, using the newer methods of 3-dimensional reconstruction from serial sections, introduced chiefly by Sjöstrand. Cohen reported that he is studying serial sections of the cockroach neuropile with the electron microscope and believes it will be possible to define at least some synaptic areas on defined cells. Bullock added that the insects and their allies are particularly good for such close-grained anatomical study because their neuropile is so compact and differentiated, ranging from crude haystacks to highly ordered and repetitious fine structure. He shares the view that mapping in detail, by serial reconstruction, is a

worthwhile, feasible, and indeed urgent task, though enormously time-consuming at present.

Trujillo-Cenóz (1965) has done a detailed analysis of a selected piece of neuropile, the visual cartridge in the optic lobe of the fly, involving all the connections within a considerable volume among 20 specified neurons. That his findings have been consistent from individual to individual, with consequences for recent theories of motion perception, points to the power of the method.

Cohen mentioned another approach which is being used by Guthrie (1964) in Aberdeen: the injuring of afferents for terminal degeneration effects. Although this degeneration is not as apparent in the invertebrate as in the vertebrate neuropile, it should be possible to follow it with the EM. Luco said he has tried this without seeing any difference between the normal and operated animals; Guthrie, however, is said to find differences.

Although at the moment it seems discouraging, Kandel would like to keep in mind the electrophysiological approach to mapping, using antidromic stimulation with electrodes in two cells to show whether they are connected. Kandel, in collaboration with Coggeshall, has combined electrical with pharmacological differences, and light microscope staining qualities and electron microscopical texture to identify, map, and characterize thirty individual cells and seven cell clusters in the Aplysia abdominal ganglion. Details of dendrite branchings and contacts with other neurons, while not yet worked out, are within the ability of present methods.

Physiological as well as structural aspects of the memory trace were discussed. Even if no anatomical changes at the microscopic or ultramicroscopic level could be found, we might still find relevant parameters of molecular structure that could vary. Kandel suggested the kinetics of transmitter synthesis, mobilization, and release could be altered by experience. Other possibilities for labile processes are the excitability of postsynaptic receptors, and the steps following transduction of the chemical transmitter into the initial response leading to local response, electrotonic spread, and alteration of frequency of spike initiation at the trigger locus.

An important feature of the prevailing view is that any relevant transmission parameter altered in learning must be distributed among many particular neurons in a way specific

for the memory. Burke (1966) illustrates contemporary thinking in terms of neuronal circuit models for conditioning.

It should be added, too, that the frequently used term "switch," in abbreviated discussions of the memory trace as a connectivity change may, by conveying several different meanings, be an intellectual trap. As its use originated among neurophysiologists, it should be envisaged in the usual case as a device that can be made to alter the transfer function in any degree, that is, to change by graded slight amounts the frequency of output train impulses to a given input train. Switches are probably relatively seldom fully closed or open and synapses are usually used to carry streams of arriving impulses to influence bursts, trains, or streams of outgoing impulses.

Considered in this light, the proposition seems supportable that the memory trace mechanism for learned behavior is the same as that for phylogenetically fixed patterns (i.e., instincts) in terms of physiological properties, the anatomical basis for connectivity, and the degree, sign, and timing of mutual influence between nerve cells. Therefore we can say with respect to learning that establishing the basis for the stored patterns of instinct will not help to establish which of the many determinants of connectivity and interaction actually undergo change, or whether they are the same for all kinds of learning and stages of consolidation.

The problems of where the altered loci that form the neural basis of a memory trace are distributed have distinguishable aspects each with its own interest. One can be stated thus: Given the minimal essential population of neurons for a learned action, where among these are the altered loci? This is the basic question of location of the engram without the complication of redundancy. But there is reason to believe redundancy of some sort enters at least in higher animals. This poses additional questions: Is the engram reduplicated in widely separated parts of the brain as Lashley concluded? Even if not, how is it distributed among a mass of supposedly largely redundant neurons? What is the role of probabilistic operation in large populations involved in the decision-making stages?

The Work Session touched upon the fundamental neurological question of probabilistic vs. deterministic operation of higher nervous centers but could not deal with it in depth; this report will likewise not attempt a significant treatment of the problem, and the reader is referred to some discussion

provided by Bullock (1957,1965); Brazier (1960); and Adey and Walter (1963).

Role of the Neuron Soma

Hoyle triggered a general discussion of the significance of the perikaryon or soma when he replied to Schmitt's question, "Where is learning in terms of the neurons?" Hoyle asserted that one could start by ruling out the cell bodies, at least in insects, because all transactional events occur in the dendritic elements. The perikaryon is there to supply trophic support and to keep the transactional and propagating branches alive. Strumwasser noted that neurons are reputed to exist which have an electrically excitable cell body that in normal life probably never experiences an action potential, i.e., is never invaded through the single-stem process from the axon and dendrite branches.

Wilson thought it necessary to identify a neuron that has actually learned, and then to determine whether the cell body is necessary for learning to occur. This could be done in crayfish, since its motor neurons have been studied and their cell bodies can be removed without too much violence to the ganglion. A positive result, that memory survives loss of the relevant somata, would be clear-cut; but a negative result might simply mean the wound disrupted performance though the memory trace remained.

Cohen stated that the role of the soma should not be underestimated even in those neurons in which electrical activity cannot be recorded; one should be cautious in saying that such cells are not invaded electrically. They must also be responsible for some metabolic processes resulting in the production of substances involved in synaptic transmission in the neuropile, and could therefore be used for many metabolic studies. Kandel suggested that the metabolically interesting change in the network may be in the presynaptic neuron.

As though to illustrate the experimental utility of the soma, Tauc brought up a preparation employing the soma of cells in Aplysia that is useful for studying the potentially important phenomenon of desensitization to transmitter. In Aplysia, cholinergic receptors are located on the soma of some cells. If ACh is injected onto this membrane, desensitization occurs, just as it does when transmitter is applied at the synapse and stimulation is repeated (Tauc and Bruner, 1963). One can measure desensitization by the increase in the strength of stimuli to multifiber preganglionic nerve that is needed to

produce a given height of postsynaptic membrane potential change after ACh has been applied in a quantity small enough to produce no permeability change. There is a drastic effect on the efficacy of the test shocks but the system's sensitivity to the drug decreases for about 20 to 30 minutes. During this test there is no reflection in the biophysical constants of the membrane.

Role of RNA, Protein, and Certain Molecular Species

A considerable amount of attention has been directed at a possible role of RNA, DNA and protein biosynthesis in the learning process. The earlier literature has been reviewed (Chamberlain, Rothschild, and Gerard, 1963; Dingman and Sporn, 1964; Gaito, 1966). A notable feature of the hypotheses to date is the absence of definite propositions as to the way these molecules might actually be involved.

The work of Agranoff and Strumwasser, aimed at testing whether RNA may have a role in learning, has been mentioned above. Agranoff presented a speculative model of the effect on learning of puromycin blocking of protein synthesis (Section V, above). It is based on the time factors observed in the study of puromycin effects as well as the classical conditioning requirement that CS must precede the US. The model resembles a tape recorder with a short-term store, a long-term store, a recording head, and an erasing head. Recording and erasing occur constantly. A second recording head simply transcribes from the short-term tape to the long-term tape. It would seem, he suggested, that puromycin affects transcription from some sort of short-term store to a long-term store. Agranoff is concerned with further biochemical experiments. In addition to protein inhibitor studies, he suggested trying to specify anatomically specific relevant events by using localized injections over different parts of the brain followed by autoradiography.

Crain and Bornstein's application of immune allergic encephalomyelitis serum to nervous tissue cultures has not yet been applied to the study of learning, but Schmitt felt this is of great potential interest. Since the action potential in an axon can be blocked by application of antibodies to axoplasm, it should be possible to develop specific antibodies against specific proteins which could be used to determine further the significance of protein in learning.

Schmitt described his own work with Huneeus-Cox (Huneeus-Cox , 1964; Huneeus-Cox et al., 1966; Schmitt and Davidson,

1965), in which no less than 14 antigens were found in squid axoplasm, from which one may obtain 6 antibodies. This mixture of antibodies, when injected into the axon under specified conditions, blocks action-potential propagation without alteration of membrane potential. The protein involved has not yet been isolated but this will probably be done.

According to Schmitt, there is a family of soluble acidic proteins in neurons, globular proteins of ca. 25,000-50,000 molecular weight. The combination of these proteins in quarternary conformation depends on extraordinarily small changes that might cause them to interact. Thus these globular proteins might form filaments or fibers by change of one amino acid residue. In principle, at least, they might form the so-called structural protein of a membrane by similarly miniscule alteration of the molecule. The nerve membrane clearly does involve a structural protein, the nature of which remains to be identified; in properties it may resemble that of mitochondrial structure proteins. A mosaic of such protein molecules in two dimensions could represent a code, a unique aggregation of determiners in a plane that might be the neuronal membrane itself or at a synaptic interface might determine the function of the synapse. Schmitt emphasized that the current concept of the synapse, in which there is a presynaptic fiber, a terminal, a postsynaptic fiber, and a cleft (an aqueous site where neurohumor is poured out when the impulse arrives), is over-simplified in failing to take account of the role of macromolecules, chiefly protein, which occur in the cleft and are associated with synaptic membranes. Crain's model system may provide an opportunity to test Schmitt's idea; namely, to clarify the role of protein in synaptic function. If puromycin were applied to a CNS tissue culture, and if it in fact delayed or even inhibited completely the formation of synapses, it might provide an indication of a way in which protein works.

Schmitt recalled J. Z. Young's saying that learning is in nets and not in cells. This seems to implicate the difference between nets and cells, i.e., the junction. Schmitt suggested that a recognition protein may exist that is characteristic for neurons; it is this for which he is looking. He emphasized that coding may involve a more sophisticated concept than that RNA as RNA is somehow coding behavior in an enormous net. If, however, one accepts the idea (as clearly we must) that to put information into molecules we need polymers, then the polymer must have a minimal number of monomers and a conformational variance that permits this information to be stored and to be read out. To Schmitt, the possibility that small molecules may change the quarternary conformation

of these monomers is strongly suggested by the experiments of Curtis and others. Schmitt admitted that Curtis himself probably would not interpret in this way his results showing that amino acids can change the hyperpolarization at synapses.

Schmitt proposed that the very simplest systems are relevant, though admittedly the phenomenology might be different from that of higher forms. He referred to viruses and their protein coat (essentially a monomolecular layer of globular proteins), saying that it is not inconceivable to him that molecular neurology might be practiced at this level. For example, the empirical discovery that the application of cytotoxic drugs permits transplantation of organs by muzzling the immunological recognition system clearly shows that the molecular concept, once grasped, can be applied to problems of lasting characteristics of cell membranes.

Cohen envisions a presynaptic impulse as altering the synthesis of RNA in the postsynaptic cell, which in turn is responsible for increased synthesis of the enzyme necessary for transmitter development. He sees this as a quantitative phenomenon; namely, the production of greater amounts of a substance already present. Cohen asked Schmitt to elaborate on the notion that there is yet another way in which use, in a postsynaptic cell, can be linked with protein production.

Schmitt explained that at the NRP Work Session on The Synapse (Robertson, 1965) Bodian demonstrated endings on dendrites which, although remote from the endoplasmic ribosomal system, showed regular Nissl substance gathered around the postsynaptic ending. At that meeting, Gray said that on most postsynaptic endings a substance that seems to be RNA tends to accumulate opposite the ending, this again being remote from the biosynthetic center of the cell. They interpret these accumulations as being the sites near the ending where some substance that facilitates the flow of the impulse across the junction is synthesized. Curtis obtains a strong change in membrane polarization by injection of d-glutamic acid upon a synapse in the cortex; Lipmann finds that acidic amino acids inhibit protein synthesis in slices of rat cortex; direct electrical stimulation also inhibits synthesis. Consequently, Schmitt believes that liberation of compounds of this kind, in the cortex at least, could change the biosynthetic patterns (proteins). Strumwasser suggested that Bodian's sites might serve to destroy the transmitter.

Bullock emphasized that in most animals, the site in the cell body of protein production is far away from the pre-

sumed locus of stored information. Schmitt said that while the protein produced at the terminal is unknown, the soma is the site of synthesis of protein that moves down the axon. Cohen did not consider the axon's length to be critical, since he finds that when a neuron is injured near the end of the axon, changes soon occur in the soma. Schmitt agreed that information of this kind is passed fairly rapidly along axons.

Eisenstein suggested that the experiments done by Eccles (Buller et al., 1960; Eccles et al., 1962) and by Weiss (1941) on modulation and myotypic specificity may be important in uncovering axoplasmic processes that may play a role in the modifiable behavior such as learning. Eccles has crossed motor fibers to red and white muscle in young cats and changed the electrical and biochemical specificity of the muscle. Eisenstein assumes that both types of synapses are ACh-mediated or, if not, that this effect is due not to transmitter alone but to some X-substance moving down the axon. Weiss has shown that by crossing motor fibers of flexor and extensor muscles, he can cause changes in their central connections. Eccles, in turn, has recorded postsynaptic potentials in the cord and shown that when flexor and extensor motor neurons are crossed, apparently the proprioceptive feedback from these muscles is changed. To Eisenstein, the evidence is more than suggestive that changes in the cord result from postsynaptic changes across the neuromuscular synapses.

Agranoff called attention to the subject of phospholipids, which he thought should be mentioned because as the other, non-protein part of the neuronal membrane, they might have a part in coding. Since unit membranes can be mosaic structures, changes in charge of the phospholipids might change the synapse in some way that involved recognition.

Schmitt compared the situation in study of learning today with the early days of study of muscle physiology. At one time it was assumed that lactic acid caused muscle contraction since contraction stopped in anaerobiosis; if oxygen were added, the "oxygen debt" was supposedly paid immediately. Therefore, it was assumed that, in fact, lactic acid pulled molecular triggers in muscle to make it contract. Because of this wrong inference, people started to draw models in which elongate proteins collapsed as a function of pH, etc. Then came phosphagen and ATP and the search for what was called the "primary process" in muscle; the physical process causing contractility, as contrasted with slower recovery processes, was pushed further and further back. We now know that muscle contraction depends on interactional parameters between two

specific proteins, myosin and actin, and that conformational factors are involved. The delayed energy-transferring reactions do not in the first instance produce the overt phenomenon. The lessons learned from the history of muscle physiology and its conceptual revolutions can be applied to the problem of learning and permanent storage today: the primary immediate process should be identified if possible and disentangled from associated processes including those of energy transfer.

Kandel replied that perhaps 20 years from now someone will use this example to describe work done today, but in the meantime our imperfect pathways are still better than the ones of years ago.

Role of D-C Potentials

Quarton brought up the subject of d-c potentials and asked whether work with an electrode in one part of a ganglion might show that shifts of d-c potential can be significant in selection of certain pathways for learning. According to Kandel, he and Tauc have tried passing current between the top and bottom Aplysia ganglia, but their results have been inconclusive because of technical problems. Earlier work, for example, by Auger and Fessard (1929) and Hughes (1952), has shown that crude overall polarization of a ganglion in an intact insect alters the flexion-extension movements of the legs in a coordinated quasi-normal way, and in the opposite sense for the two polarities. This suggests that current-direction-sensitive parts of the neurons are not oriented haphazardly and that the general fields of d-c in the tissue might be influential in favoring some output patterns in certain cases. Tauc expressed his belief that such a polarizing current could cause an increase or decrease of the synaptic input. When asked by Quarton if one could look for d-c shifts regionally as a consequence of selection of one pathway rather than another, Kandel replied that he and Tauc believed presynaptic interaction to be responsible in Aplysia. Although he cannot specify what is happening in the presynaptic terminal, by analogy with the effect of polarizing currents at the neuromuscular junction, he would argue that perhaps hyperpolarization of the terminals is occurring. By passing polarizing current, one may be hyperpolarizing terminals that happen to be located in the field, and therefore increasing the amount of transmitter that may be located there. There is reason to believe that at neuromuscular junctions transmitter mobilization is a function of the terminal potentials and that some of these effects persist for some time after the current is

turned off. Schmitt suggested that if the experiment were done and the distribution of current were such that current flowed through the cell, the negatively charged acidic proteins which preponderate in neuroplasm would be transferred in the direction of the membrane and possibly be deposited on it; the substances mentioned by Agranoff (which were almost all electrolytes and many of them cations) would be moved in the opposite direction. It is therefore possible that specific molecular information might be involved.

Time Factors in Memory; Consolidation

Several distinct time factors are involved in learning, some of them peculiar to certain subclasses of learning. Only a few were explicitly discussed at the Work Session. The question arose whether in order to call a given behavior modification "learned," there must be a minimum duration of persistence of the modification. The psychologists were generally unwilling to set an arbitrary minimal duration of memory. They preferred to think of there being both short-lasting and long-lasting memories.

Bullock, however, believes that when people commonly use the words "memory" or "learning," the phenomena are usually thought of as having a relatively lasting quality on some appropriate scale. Regarding the time scale, he referred to the elegantly simple preparation of the isolated crayfish stretch-receptor neuron with its single synaptic input, an inhibitory axon. When suitably stretched, this sensory neuron fires "spontaneously" at a predictable rate. The intervals between firings are altered if an inhibitory impulse is elicited by a stimulator controlled by the last sensory neuron spike after a preset delay (contingent reinforcement) and this effect is different if the inhibitory input is non-contingent. This example would seem to satisfy all the criteria enunciated for instrumental conditioning, but the time scale is so short that almost no effect remains one second after the last "reinforcing stimulus." We can hardly apply the term "learning" here, but this common-sense criterion for learning does not appear in the definitions defended.

Another situation in which a semi-arbitrary time scale is essential is in the distinction between one-trial learning (e.g., a bee finds food in a certain place and returns to that place; a rat refuses to step on a grid that once gave it a shock) and simple innate, directed responses to stimuli (unconditioned responses). The minimum retention time, and hence the distinction, is not quite arbitrary, but is based on our

general experience with similar animals. Just as a hawk may (presumably instinctively) direct its movements for some rather short time period toward the site of a brief movement (stimulus not maintained or recurring; no reinforcement), the bee and the rat direct their movements for a longer time (hours or days) relative to a brief stimulus even without recurring input or reinforcement. This is not to argue that the two are equivalent or that the apparently arbitrary time difference is not significant. Bullock agrees with Lorenz that there is a fundamental difference between behavior fixed by evolution and that fixed by experience, though mixtures of them are common.

Schmitt asked for a discussion of the time parameters related to fixation or consolidation of memories. McCleary presented the following figures for the time required in several cases although he first expressed the opinion that this is not critical for the study of memory: Consolidation takes 15 to 20 minutes in the (1963) hamster experiments of Chamberlain, Rothschild, and Gerard on spinal cord retention. Deutsch and Deutsch (1966), after reviewing studies employing electroconvulsive shock (ECS) as an amnestic agent, conclude that, with this technique, consolidation seems to be complete by approximately one hour. On the other hand, ether anesthesia has no amnestic effect if administered more than 5 minutes after learning, pentobarbital anesthesia can produce amnesia 10 minutes after learning, and a Metrazol convulsion is effective in producing retrograde amnesia for tasks learned as long as 4 days previously (Pearlman et al., 1961). A still longer estimate of consolidation time is provided by Flexner, et al., (1963) who produced a 6-day retrograde amnesia in mice by injecting puromycin into the hippocampus and entorhinal cortex. In man, of course, retrograde amnesia following head trauma can extend back months and even years. Estimates of memory consolidation time obtained in this way thus are seen, unfortunately, to be highly dependent upon the agent that disrupts the memory process.

Chorover found this discussion of the "duration of consolidation" to be somewhat misleading. He does not agree that by charting the temporal characteristics of memory interference produced by different agents one can specify the necessary and sufficient time for "consolidation" to occur. On the contrary, he believes all that a detailed temporal analysis of the effects of a given treatment can show is the necessary time interval that must elapse between training and treatment in order that no memory interference be produced. To him it is obviously unwarranted to conclude, further, that this in-

terval defines the time sufficient for "consolidation" to be completed. In his view of variation in the duration of retrograde amnesia produced by many different treatments, the idea of a unitary, single-stage consolidation process must be wrong. As an alternative, he suggested that different treatments interfere, more-or-less selectively, with different stages of a continuous process of indeterminate length. Because each stage of the process is presumably mediated by a somewhat different mechanism, a treatment that interferes with one stage may not interfere with another. Since the process is a sequential one, an equivalent degree of terminal interference can be produced by different agents acting at different times. As a concrete example, he noted that the duration of ECS-induced retrograde amnesia may be as brief as a few seconds (Chorover and Schiller, 1965; Schiller and Chorover, 1966b; Quartermain et al., 1965; Lee-Teng and Sherman, 1966; Paolino et al., 1966), while that produced by various drugs may be much more prolonged (Flexner et al., 1963; Barondes and Cohen, 1966; Agranoff et al., 1964,1965; Paolino et al., 1966). His point is, of course, that as we learn more about time-course and mode of action of the various individual amnestic agents, we will be increasingly able to specify the mechanisms underlying successive stages of neural information processing.

Kandel also protested that all these periods of learning are defined by periods of amnesia; this, however, is only one dimension of the duration of learning. Hoyle felt it was meaningless to discuss absolute terms because of the varying lengths of normal life spans of lower animals. He said that in principle, we are not concerned with whole animals at all but with a process that can occur in a bit of nervous system.

Agranoff pointed out that goldfish retain some tasks and not others; even in the tasks involving what is called "permanent memory" there seems to be a short-term and long-term component, which he has shown can be chemically separated. There was some discussion of the significance of convulsive shock in studies of memory. Chorover noted that unless a given experiment includes control for possible aversive effects of an amnestic treatment, it is unwarranted to conclude that behavioral disruption is due to memory interference. Such controls provide the only basis for resolving a controversy which has plagued this research area for many years. Agranoff reminded the group that he obtained memory loss with pre-trial puromycin injection, as well as with ECS.

Strumwasser cited the work of Lee- Teng and Sherman (1966)

a one-trial learning situation in which a chick pecks at a sponge or piece of cotton filled with water and learns to avoid the sponge thereafter, until he is given gas anesthesia, after which he forgets his aversion to the sponge. Strumwasser interpreted this effect as perhaps producing a block in pathways that must be active for a certain length of time before information can be stored; he feels that protein synthesis may be only coincidentally involved. Agranoff expressed some concern about the controls in that experiment.

Strumwasser took issue with Agranoff's statement that he had demonstrated some interference with memory, saying that Agranoff might be interfering instead with information processing. There are many possible mechanisms that could be disturbed without reaching the storage system. Agranoff explained that he considers "fixation of memory" as an all-inclusive term. Chorover said that the tendency to talk about short-term (processing) and long-term (storage) mechanisms sets up a misleading dichotomy between sequentially related events along a continuum. What is clear is that at the start of training, certain items of information (stimuli and/or relations between them) are absent from memory. Training consists of assimilating and/or relating these items. After training the items are present in memory, in some form. There are hunches based on electrophysiological and biochemical data, that there must be a sequence of events related to this process. Surely neuroelectrical events cannot occur without some sorts of information-processing changes indicated by the biochemical events; but even if they could occur alone, they could not account for long-term storage. One can interfere with the electrical activity of the brain in a number of ways without disrupting long-term retention; but interestingly, relatively recent events tend to be uniquely vulnerable when one interferes with neuroelectrical activity.

Chorover suggested that there are no discrete steps between the transient electrical changes that accompany stimulation, and the subsequent more-enduring complex neurophysiological pattern of activity that finally leads to some lasting structural change. Rather, there must be subtle transitions. It is quite possible that the initial electrical events set up conditions for the long-term changes, which themselves are insensitive to interference, for example by ECS. One does not thereby reconcile the time differences in effects of ECS and puromycin by assuming that the first affects "short-term", and the second, "long-term" memory. What seems more fruitful is to infer the existence of a continuous sequence of closely related events which eventually results in more-or-less permanent

memory storage. The interesting area to study is the potential transition zone from transient neuroelectrical activity to biochemical storage. Even the biochemical activity takes time, which suggests that the times involved in protein synthesis are relatively prolonged.

This differentiation was accepted by Schmitt, who said that it is tacitly assumed that ECS does have its effect on propagation of impulses in nets; but he would like to see the evidence. He does not know of an experiment in which the equivalent of an ECS had been tried on firing neurons in a simplified or partial system, in which some parameters can be controlled. In such a relatively simple system as the tissue culture of Crain, in which there are evoked potentials with characteristics of flow of action waves in nets, Schmitt would like to know the effect of imposed voltages similar to those seen by the nets in the brain as a result of ECS. Kandel said that people have studied quite precisely transmembrane changes during convulsions, and one would not expect this to be different in a simplified preparation. Quarton suggested, on the contrary, that in ECS, local effects are compounded at the system level. Agranoff felt that another convulsive agent would probably do the same as ECS, causing everything to fire (with utilization of ATP and glycogen), and making the amount of energy for other activities rather low for a period of time; this energy depletion could account, in a crude way, for some decrease in protein synthesis.

Strumwasser stated that one cannot resort only to biochemical analysis to study fixation time; he knows of no way to find out how the information process is being disrupted without using some electrophysiologic indicators. Chorover asked if anyone has identified enduring electrophysiologic consequences with a reasonable time course. If one assumes that information processing may go on in neural nets for a period of one-half hour or more, there should be some electrical evidence for this. Strumwasser pointed out that Hoyle has been able to sustain the output of an axon for long periods of time, 2 hours or so, at a much higher level than control value.

Chorover considered this an example of the difficulty of using single units as models of learning. If one has a unit in which some enduring change in output activity has been produced, the unit is now occupied with that activity and cannot be available for additional inputs that might be behaviorally significant. Several voices were raised in dissent to this statement. Chorover explained that he was actually expressing the feelings that Deiter's cells, early in the sensory pathway

for vestibular input, are an unlikely place to look for changes specifically related to information storage. How could subsequent information be incorporated if these changes had occurred peripherally? Or indeed, how can any information be acquired sequentially on unrelated tasks? Strumwasser answered that a change's occurrence in a sensory pathway does not mean that it is part of the learning pathway; there may be many changes going on. The problem is to tell which ones are most relevant.

X. CHAIRMAN'S SUMMARY

At this Work Session several actual and potential biological preparations were considered and compared as convenient vehicles for the study of learning mechanisms. The preparations discussed ranged from paramecia to the lower vertebrates, although insects (particularly locusts and cockroaches) and molluscs (Aplysia) received the greatest attention. Although in one instance a whole organism was used (goldfish), and in another, a tissue culture (neonatal mouse cortex), by and large the preparations consisted of surgically isolated partial systems.

This Work Session, like most NRP Work Sessions, served to focus attention on problems rather than to solve them. Among the issues raised were the following:

1. Is there a definition of learning to which most psychologists will agree, and if so, what is it? The psychologists appeared to agree that they disagree; there are many definitions, but none has become canon. This is partly because it is generally recognized that several distinct phenomena are embraced by the term, each more readily defined than the whole and each regarded as a type of learning. The most satisfactory definition among a number considered is that learning is the process by which an activity originates in or is changed through reacting to an encountered situation -- provided that the characteristics of the change cannot be explained on the basis of native response tendancies, maturation, or temporary states of the organism such as fatigue, drugs, acclimation, disease, or alterations in the receptors or effectors (slightly modified from Hilgard). The most satisfactory view includes as types of learning: imprinting, one-trial learning, insight learning, latent learning or exploratory learning, habituation, classical conditioning, and operant conditioning. Although the outer boundaries of what should be considered learning may be in dispute, the demonstration of conditioning, either classical or instrumental, is generally accepted to be a bona fide example of learning in whatever organism it occurs. Difficulty often arises in the critical use and specification of acceptable operational criteria for applying the term "conditioning." A detailed exposition of these

criteria has been provided in this report for the guidance of experimental design.

2. Is there a specific phylogenetic point at which learning occurs? The answer to this obviously depends upon one's definition of learning. However, despite reluctance to specify an arbitrary point, the psychologists tended to agree that learning in the form of conditioning occurs unquestionably at the level of the annelids. While it may possibly also occur below that point, our ignorance of the behavioral repertoire of the animals studied, coupled with our inability to empathize with organisms considerably unlike ourselves, makes convincing behavioral experimentation extremely difficult. The equivocal reception of planarian studies serve as a case in point. This is the most cautious position represented. Some participants, however, while not satisfied with the demonstrations to date, believe on balance that planarians and even sea anemones and some protozoans exhibit plasticity within the broader definitions of learning.

3. Is there a physically minimal system in which learning occurs? Again, one's definition of learning is critical. The evidence permits the statement that for operant conditioning the minimum number of neurons that need be involved must be smaller than a few hundred in insect ganglia and it may well be only two or three neurons in Aplysia ganglia for either operant or classical conditioning.

4. What preparations have been used with promising results? Those discussed at this meeting include the isolated ganglia of the marine gastropod Aplysia; the isolated thorax or neurally isolated single segment of insects (cockroach, locust); the neurally isolated spinal cord or the decerebrate brain stem and cord of lower vertebrates and even mammals; tissue cultures of sizeable fragments of cortex from young mammals (neonatal mouse); and, for certain drug experiments, the intact fish.

5. What additional preparations appear to be worthy of development? In the opinion of some, the ciliate protozoans, the coelenterates, and flatworms

merit continued attention particularly considering our improved insight into their normal environmental stimuli and behavioral repertoire. While these forms cannot readily be studied by present techniques of intracellular physiology or by the isolation of quantities of pure nervous tissue, some may have special advantages, for example, their tolerance for being whittled down. Together with other lower groups such as echinoderms, rotifers, nematodes, nemerteans, the simpler and sedentary pelecypods, annelids, crustaceans and the like, these lower species could give us a greatly improved perspective on the types of learning and their properties and requirements in more primitive versus derived forms. Comparative phenomenology is much needed to guide the physiological and biochemical studies. Preparations ready for new physiological examination with the use of learning paradigms include ganglia of annelids (polychaetes, leeches, earthworms), ganglia of a range of gastropods from the wide diversity available, ganglia of the peripheral organs of cephalopods (for example, the arms), and parts of vertebrates such as the retina and spinal cord.

6. What do the neurophysiological and chemical studies to date suggest regarding the mechanisms involved in learning? Are memory traces widely diffused or localized, redundant or unique, basically synaptic or involving other parts of the neuron, primarily due to chemical or to anatomical alteration? Are they the same for apparently dissimilar forms of learning? Do the physical correlates of a given type of learning differ from species to species? The questions raised are an indication of some of the issues recognized at present! In addition to those above, the alternatives of motor versus sensory tapes form one active issue. Another issue is whether instinct and learning are basically similar or different in storage mechanism. The question of whether memory traces involve structural change or maintained activity change appears to be evolving in its formulation. The issue of redundancy can be raised at microscopic level within a limited group of neurons as well as at the level represented by Lashley's reduplication. The meaning of structural alteration has expanded to several levels: those of the light microscope, the

electron microscope, and molecular configurations. It can now include the small shifts in normal features that determine threshold, summation, facilitation, transmitter mobilization, release, and time course, spike frequency, and the like. The role of RNA, protein or particular molecular species remains to be explained, and the possible role of d-c potentials was little more than mentioned. The issue of placing limits on the time span relevant for learning was somewhat clarified by discussion of extreme cases.

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